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IODINE IN RELATION TO PLANT NUTRITION[1]

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INTRODUCTION

Although investigations dealing with the effects of iodine on plant growth have been reported over a period of some 40 years, the exact relation of iodine to plant nutrition remains to be determined. Interest in this problem is augmented by the importance of iodine in animal nutrition.

The writers' approach to the problem of the importance of iodine in plant nutrition has involved two distinct lines of study. First of all, critical experiments dealing with the question of the essential nature of iodine in plant nutrition have been conducted. The knowledge as to whether occasional stimulations of plant growth by iodine additions to soils are due to the remedying of a deficiency of an essential nutrient or to some secondary effect of iodine is of prime importance to a rational consideration of the problem. A second line of approach has been based on the assumption that responses to iodine additions to soils are most likely to be found in regions where simple goiter is endemic. Oregon is in such a region (*5*).[3]

REVIEW OF LITERATURE

Several investigators (*14*, *21*, *22*)[4] have obtained stimulations of plant growth from additions of iodine to sand and solution cultures. White (*23*) found that iodine, as well as manganese, zinc, and boron, appears to be essential for the growth of excised tomato roots. Numerous failures to obtain stimulations by nontoxic additions of iodine-bearing compounds to sand and solution cultures have also been reported (*4*, *16*, *19*). Iodine levels of the order of 1 or 2 parts per million are generally toxic to higher plants, except halophytes.

Growth responses of micro-organisms to iodine treatments have also been reported. Several workers have reported stimulation of *Azotobacter chroococcum* (*6*, *10*, *21*), *Saccharomyces cerevisiae* (*7*, *10*), and *Bacillus subtilis* (*10*) by iodine treatments. Ishikawa (*9*), however, found that the activity of proteolytic bacteria was repressed by as little as 20 p. p. m. of iodine. These effects were obtained with

[1] Received for publication February 19, 1941. The investigations herein reported were carried out as an Adams project. The data are taken from a thesis presented by the senior author to the faculty of the Graduate School of Oregon State College in partial fulfillment of the requirements for the degree of doctor of philosophy. Published as Technical Paper No. 374; contribution from the Soils Department.

[2] The authors gratefully acknowledge the cooperation of Dr. J. R. Haag in furnishing facilities for iodine determinations, and of Dr. W. B. Bollen and Dr. C. P. Hegarty in furnishing facilities for the microbiological work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 636.

[4] REGEIMBAL, L. O. THE ROLE OF IODINE IN PLANT NUTRITION. 50 pp. Minneapolis, Minn. 1929. (Thesis, Minn. Univ.)

iodine concentrations well below the toxic levels, since for microorganisms relatively high concentrations are required to produce toxic effects. Such growth responses are to be distinguished from the stimulations frequently given by poisons at concentrations just below toxic levels. Thus Branham (*1*) investigated 16 antiseptics, including tincture of iodine, and found that most of them had a stimulating effect at sublethal concentrations. The latter phenomenon is related to the irritability of protoplasm and must be carefully distinguished when the question of the essentiality of a nutritive factor arises.

Since even in solution cultures the reasons for growth responses to iodine additions are not clear, it is not possible at present to interpret satisfactorily the responses sometimes obtained when iodine is added to soils. Numerous cases of growth responses are recorded. Not all of these experiments may be dismissed as being statistically unreliable. As would be expected, there are also numerous reports of failures to obtain responses. The effects of iodine treatment of soils on plant growth and on the iodine content of plants, as well as the distribution of iodine in nature, have been reviewed by McClendon (*15*) and others (*13*, *17*).[5]

THE QUESTION OF THE ESSENTIAL NATURE OF IODINE IN PLANT AND MICROBIOLOGICAL NUTRITION

MATERIALS AND METHODS

Analytical Methods

The analytical methods employed varied with the type of material to be analyzed and the anticipated iodine content. Water samples were evaporated under alkaline conditions, followed by low temperature potassium hydroxide fusion. Soils and high-iodine plant materials were fused with potassium hydroxide. Low-iodine plant materials were burned in the Kolnitz-Remington (*11*) oxygen torch. The gases were absorbed in alkaline solution, the solution evaporated, and the residue fused with potassium hydroxide. Iodine was recovered by the distillation method of Bratton and McClendon (*2*), followed by bromine oxidation and thiosulfate titration. In some of the earlier analyses, iodine was recovered by alcohol extraction of a potassium carbonate paste (*8*) or a saturated solution of potassium carbonate (*18*).

Numerous blank determinations were run as a precaution against the ever-present danger of iodine contamination. The results from these blanks were usually small in comparison with the actual determinations. The recovery of iodide added to hay samples was found to vary from 90 to 100 percent. The analytical figures presented are usually the average of two or more determinations.

Purification of Nutrient Chemicals

The only report found in the literature of a cultural medium especially designed to contain a minimal amount of iodine is that of Stoklasa (*22*). He gives no details of his purification methods beyond the implication that the nutrient salts were purified by recrystalliza-

[5] Karns, G. M. The relation of iodine to plant growth. 13 pp. Pittsburgh. (Mimeographed report of the Mellon Institute of Industrial Research.)

tion. Stoklasa's analyses of plant materials grown on his medium show a substantial iodine content. The writers' analyses, presented in table 1, emphasize the necessity for purifying certain of the nutrient chemicals.

Since little evidence was available as to the efficiency of removal of iodine compounds from nutrient salts by recrystallization, more positive purification methods were sought for use with the necessary nutrient chemicals. Where possible, the maximal iodine content of the chemical after purification was approximated by studying the rate of removal of iodine during the purification procedure. Thus in the purification of dipotassium phosphate, a saturated solution of the salt was repeatedly extracted with ethanol. The progress of iodine removal was followed by analyses of the successive alcohol extracts.

It is estimated that the procedures followed are sufficiently effective to reduce the iodine content of the major nutrient chemicals to below 1 part per billion; that of water to below 0.001 p. p. b. The resulting nutrient solutions should have an iodine content of the order of 0.001 p. p. b. Such chemicals and solutions are hereafter referred to as "iodine-free."

TABLE 1.—*The approximate iodine content of some chemically pure chemicals and other substances*

Chemical	Iodine content	Chemical	Iodine content
	Parts per billion		*Parts per billion*
Dipotassium phosphate	25	Magnesium sulfate	18
Monopotassium phosphate	16	Ferric tartrate	100
Phosphoric acid	>2.5	Ammonium hydroxide, on the basis of NH_3 content	5
Potassium carbonate	20	Sucrose, household	>3
Potassium hydroxide	15	Starch, household	15
Potassium sulfate	25	Acetone	.8
Potassium nitrite	3,000	Ethanol	.2
Sodium nitrite	200	Carbon tetrachloride	<.1
Sulfuric acid	<2	Carbon dioxide, compressed technical grade, on the weight basis	<2
Calcium sulfate	140	Water, Corvallis tap	.35
Calcium hydroxide, technical grade	50	Water, laboratory-distilled	.005
Calcium carbonate	1,000	Water, redistilled from alkali carbonates	<.001
Calcium lactate	1,000		

Dipotassium phosphate and *potassium carbonate.*—A saturated solution of the salt was extracted repeatedly with iodine-free ethanol prepared as described below. When analyses of the extracts showed a reduction of the iodine content of the salt to less than 1 p. p. b., the solution was boiled for 30 minutes with 0.1 percent of hydrazine sulfate to reduce iodates. After reextraction with ethanol, the solution was evaporated to dryness and heated to destroy excess hydrazine.

Sulfuric acid.—The acid was boiled to about two-thirds of the original volume.

Calcium sulfate, magnesium sulfate, and *ferric sulfate.*—The finely divided salt was suspended in two times its volume of chemically pure sulfuric acid and boiled to dryness.

Ammonium hydroxide.—Ammonia was purified by distillation from potassium carbonate, bubbling through potassium carbonate solution in a series of gas-washing bottles, and absorption in iodine-free water.

Nitric acid.—Chemically pure nitric acid was repeatedly distilled from barium nitrate, only the midfraction being saved. Acid several years old was used to ensure complete conversion of iodides to iodates. Owing to the difficulty of determining traces of iodine in the presence of large amounts of nitrates, this purification procedure could not be critically checked.

Sucrose.—The method of Harvey (*8*) involving repeated recrystallizations from ethanol was followed.

Ethanol.—Ethanol was readily freed from iodine compounds by distillation from potassium hydroxide after an initial refluxing period. The ethanol used for cultural purposes was redistilled.

Water.—Purification of ordinary distilled water was accomplished by redistillation from alkali carbonates in a pyrex glass still.

Cultural Methods

Two groups of experiments were conducted with higher plants. The culture solutions used in the first group were prepared from ordinary chemically pure salts and ordinary distilled water; only nominal precautions were taken against iodine contamination. These experiments are referred to hereafter as "preliminary." The solutions used in the second group of experiments were prepared from the specially purified salts and redistilled water, and rigorous precautions were taken against iodine contamination.

The basal nutrient solution used in the preliminary experiments and the cultural conditions have been described elsewhere (*12*). In all of the cultural work iodine treatments were added as potassium iodide. Stoneware and mason jars were used as cultural vessels. The seedlings, after germination in washed quartz sand, were transferred to the cultural solutions. They were held with tufts of cotton in varnished plywood covers.

The nutrient solutions used in the second group of experiments were prepared from iodine-free nutrient chemicals and redistilled water. The chemically pure salts used for supplying minor elements were not subjected to purification procedures. The nutrient solutions had the following composition: Calcium sulfate, 0.002 M; magnesium sulfate, 0.001 M; dipotassium phosphate, 0.002 M; ammonium nitrate, 0.005 M; boron as boric acid, 0.5 p. p. m.; manganese as sulfate, 0.5 p. p. m.; zinc as sulfate, 0.05 p. p. m.; copper as sulfate, 0.02 p. p. m.; and molybdenum as ammonium molybdate, 0.05 p. p. m. The pH was adjusted weekly to 5.5 to 6.0 with ammonium hydroxide or nitric acid. Iron was added as ferric sulfate at the rate of 1 p. p. m. every 3 days during the early growth stages and weekly during the later growth stages.

The success of such experiments depends on careful precautions against contamination of the reagents, nutrient solutions, and plants. In the case of iodine this is especially important because of its wide distribution. The glass culture vessels, glass-stoppered bottles used for storing reagents, and pipettes and graduated cylinders used in preparing the solutions were thoroughly soaked in acid cleaning mixtures and rinsed well with iodine-free water. The interference of iodine contamination of the solutions was reduced as far as possible by avoiding forced aeration and by using concentrated, infrequently renewed culture solutions.

The medium used for the culture of *Azotobacter agile* was prepared from iodine-free salts, and precautions were taken against iodine contamination. The medium had the following composition: Magnesium sulfate, 0.0084 M; dipotassium phosphate, 0.0019 M; calcium sulfate, 12 p. p. m.; ferric sulfate, 5 p. p. m.; boron, manganese, zinc, copper, and molybdenum as given above; potassium carbonate to pH 7.0; and ethanol, 1.1 percent by weight. The inorganic constituents were dissolved in iodine-free water. Aliquots of 140 ml. were distributed into 500 ml. Erlenmeyer flasks, which were stoppered with cotton and autoclaved. Ethanol was aseptically pipetted into the flasks after sterilization. The inverted-sucrose medium used in the preliminary experiments had a similar inorganic composition.

EXPERIMENTAL RESULTS

Preliminary Plant Experiments

A number of preliminary experiments were conducted with ordinary c. p. chemicals and tap or distilled water to determine the effect of added iodine. The results obtained in certain of these experiments are reported in table 2.

TABLE 2.—*Yield and iodine content of corn, barley, and head lettuce grown in unpurified solution cultures containing small quantities of iodine*

Iodine added to solution (p.p.m.)	Corn			Barley			Head lettuce		
	Mean dry weight of tops at 60 days	Iodine content, dry basis	Fraction of added iodine absorbed	Mean green weight at 60 days	Iodine content,[1] dry basis	Fraction of added iodine absorbed	Mean green weight at 60 days	Iodine content, dry basis	Fraction of added iodine absorbed
	Grams	*P. p. m.*	*Percent*	*Grams*	*P. p. m.*	*Percent*	*Grams*	*P. p. m.*	*Percent*
0.0	17. 2±2. 0	0. 05		26. 8±2. 5	0. 20		44. 4±2. 1	[1] 0. 27	
0.05				24. 0±3. 5	15	24	51. 8±7. 6		
0.10	14. 0±1. 8	8. 6	11	23. 8±2. 1	16	12	49. 9±6. 6	8. 7	56
0.25				[2] 25. 9	90	32	59. 1±7. 0	18	58
0.50	11. 8±1. 8	58	13	[3] 20. 8	160	21	55. 1±4. 7	39	59
1	9. 9±1. 5	88	8	([4])			38. 7±4. 0	65	31
5	2. 2±0. 4	430	2						

[1] Duplicate analyses were not made because of insufficient plant material.
[2] Mean of 3 living plants; 3 plants dead.
[3] Mean of 5 living plants; 1 plant dead.
[4] All plants dead.

Additions of potassium iodide equivalent to 0.1 to 5 p. p. m. of iodine depressed the growth of corn (*Zea mays*). A concentration of 1 p. p. m. of iodine depressed growth by approximately 50 percent, while 5 p. p. m. was very toxic. The above culture solutions were made up with tap water in new glazed stoneware crocks.

In a similar experiment with barley (*Hardeum vulgare*), in which solutions made up with distilled water in glazed stoneware crocks were used, no stimulation was observed over the range of 0.05 to 1 p. p. m. of added iodine. A concentration of 0.5 p. p. m. of added iodine was definitely toxic; 1 p. p. m. was lethal.

Another experiment with barley was made to determine the statistical significance of the results from the addition of 0.05 p. p. m. of iodine. The cultural solutions were prepared with distilled water. Eighteen

1-quart mason jars were used for each treatment. The mean dry weight of the tops was 0.422 ± 0.033 gm.[6] for the control solution as compared with 0.457 ± 0.025 gm. when 0.05 p. p. m. of iodine was added. The difference is not statistically significant.

An experiment was made with head lettuce (*Lactuca sativa*), grown in glazed stoneware crocks in solutions made up with distilled water. Iodine was added at rates of 0.05 to 1 p. p. m. (table 2). The control plants were inferior to those treated with 0.05 to 0.5 p. p. m. of added iodine. The plants treated with 1 p. p. m. of iodine were slightly inferior to the controls. While the responses to individual iodine treatments are not statistically significant, if the iodine treatments from 0.05 to 0.5 p. p. m. are grouped, a slight response to added iodine appears to have been obtained. The control treatment was applied to six plants, the other treatment to four plants each.

In another experiment, head lettuce was grown in 1-quart mason jars in solutions made up with distilled water. A treatment with 0.25 p. p. m. of added iodine was compared with the control solution. No stimulation was observed. Further work with head lettuce in stoneware jars failed to show any stimulation resulting from the added iodine.

TABLE 3.—*The calculated iodine content of the control solutions used by several investigators*

Workers	Plant analyzed	Average iodine content of the control plants	Average calculated iodine content of the solution	Independent estimates	Remarks
		P. p. b.	*P. p. b.*	*Number*	
Stoklasa (*21*)	Day lily (*Hemerocallis fulva*).	420	2,500	4	
Stoklasa (*22*)	Sugar beet:				Purified chemicals and ignited sand cultures.
	Tops	640	1,000	5	
	Roots	210	2,700	5	
Scharrer and Schropp (*19*)	Sugar beet:				Sand cultures.
	Tops	1,370	25	7	
	Roots	670	55	7	
	Turnip:				
	Tops	970	55	6	
	Roots	1,150	80	6	
Orr, Kelley, and Stuart (*16*)	Peas (1)	[1] 140	35	3	Reagent-grade chemicals.
	Peas (2)	[1] 81	35	1	
Regeimbal [2]	Wheat:				Purified chemicals.
	Tops	190	50	6	
	Roots	930	70		
Lewis and Powers (present paper).	Corn	50	.6	4	C. p. chemicals and tap water.
	Barley	200	.8	4	
	Head lettuce	270	3.5	4	C. p. chemicals and distilled water.

[1] Fresh-weight basis.
[2] See footnote 4.

Iodine analyses were made of certain plant materials grown in the above-described experiments. In general, the iodine content of the plant material was greatly increased in the presence of added iodine, the iodine content being more or less linearly related to the concentration of added iodine. By assuming that plant material grown in the complete absence of iodine in the solutions would be essentially iodinefree, and assuming further that the linear relation of iodine content to iodine concentration holds at low iodine concentrations,

[6] The standard error of the mean is used in this paper.

it is possible to estimate the iodine concentration in the control solution from the iodine content of plant materials grown in the control solution and of materials grown under otherwise identical conditions in the presence of known concentrations of iodine. The data on which three sets of such calculations are based are presented in table 2. The apparent iodine content of the control solutions, as well as the results of similar calculations for the control solutions of other investigators, are shown in table 3. The apparent iodine content of the writers' control solutions in most cases is of the order of 1 p. p. b. The unpurified solutions show lower iodine concentrations than the control solutions used by the other investigators cited.

Two possible sources of error may affect the above calculations. In the first place, the linear relation between iodine concentration in the solution and iodine content of the plant material may not hold at low iodine concentrations. In general, the error would be expected to be greater, the greater the difference between the iodine concentrations in the known and the unknown solutions. That this source of error is not serious in the writers' calculations is indicated by the fact that such calculations based on three plant species grown in known iodine concentrations varying over wide ranges are in substantial agreement.

In the second place, while practically all of the iodine in the high iodine-content plant material must have come from the solution, this is not necessarily true of the iodine in the low iodine-content plant material. A substantial fraction of the iodine in such material may have arisen from atmospheric or other sources of contamination. This consideration would tend to make the calculated iodine content of the solution a maximal value.

Plant Experiments With Purified Solutions

Two experiments were conducted with tomatoes (*Lycopersicum esculentum*) grown in solutions prepared from purified chemicals and redistilled water. Glass culture vessels were thoroughly cleaned with acids, and special precautions were taken against iodine contamination.

In the first experiment, nine 3-liter soft-glass battery jars bearing three plants per jar were used in each series. To facilitate statistical treatment, individual plants were paired on the basis of initial size. A treatment of 0.1 p. p. m. of added iodine was compared with the iodine-free control. After 65 days the plants had attained green weights of about 40 gm. each. No yield response to iodine treatment was obtained, the mean difference between paired plants in favor of the iodine treatment being 0.85 ± 1.5 gm. At this time two plants per jar were harvested, leaving the best paired plant in each jar. These plants were permitted to grow to maturity (104 days). Neither fruit, tops, nor roots showed any response to iodine treatment.

A second experiment was carried out in 16 shallow pyrex baking dishes with glass lids drilled to accommodate the stems of the plants. Six seedlings per dish were started. After 30 days the plants had green weights of 6 to 7 gm. each. The mean of the differences between mean weights of plants in paired jars was 0.25 ± 0.10 gm. in favor of the plants grown in the presence of 0.1 p. p. m. of added iodine. At this time the plants were thinned to two per jar and

permitted to grow to the blooming stage. There was no response of either tops or roots to iodine treatments after 82 days of growth.

Sugar beets (*Beta vulgaris*) were grown for a short period in purified nutrient solutions in soft-glass battery jars. After 3 weeks of growth, there were no significant differences between the mean weights for the various treatments, which varied from 4.40 to 4.65 gm. Stoklasa (*22*) obtained a significant response to iodine treatment at 30 days after germination.

Iodine analyses were made of various parts of tomato plants grown in the presence and in the absence of added iodine. These analyses are presented in table 4. They indicate that under the conditions of these experiments the distribution of iodine in the tomato plant was fairly uniform.

TABLE 4.—*The iodine content of tomato plants grown in "iodine-free" solutions and in the presence of added iodine*

Iodine added	First experiment, iodine content of—					Second experiment, iodine content of 82-day-old whole plants
	65-day-old whole plants	Parts of 104-day-old plants				
		Leaves	Stems	Roots	Fruit	
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Iodine-free solutions	[1] 0.29	0.10	0.10	[1] 0.10	[1] 0.10	[1] 0.11
0.1 p. p. m	34	21	19	24	15	3.6
0.5 p. p. m						10.2

[1] Duplicate analyses were not made because of insufficient plant material.

The iodine content of the plant materials from the iodine-free cultures were found to approximate 100 p. p. b., and in one case with younger plants to be 290 p. p. b. The origin of this relatively high iodine content is a question of considerable importance. A calculation based on the volume of nutrient solution used to produce 1 gm. of dry plant material indicates that a residual iodine concentration of 0.001 p. p. b. in the iodine-free solutions could have contributed only approximately 0.5 p. p. b. of iodine to the plant material.

The small size and low iodine content of seeds in general seems to preclude a significant iodine contribution from this source. For example, a tomato plant weighing 10 gm. would receive an iodine contribution of only 0.01 p. p. b. from a seed weighing 1 mg. and having an iodine content of 100 p. p. b.

Two iodine analyses of the greenhouse air made during these investigations gave an iodine content of 0.01 and 0.04 γ [7] per cubic meter of air. Each sample was collected over a period of 1 to 3 months and therefore represents a mean air iodine content. The lowest air iodine content among those summarized by McClendon (*15*) is 0.04 γ per cubic meter.

After the completion of the plant experiments, large sheets of filter paper were suspended in the greenhouse and kept there for 72 days. Although the amount of accumulated dust was small, the iodine content of the paper increased 0.5 γ per square foot. This increase is equivalent to approximately 60 p. p. b. of iodine. It will be noted that the latter figure is similar in order of magnitude to the iodine content of the plants grown in the iodine-free solutions. However, the greenhouse air was not especially contaminated with iodine; in

[7] The microgram (γ) is 0.001 mg.

fact, it was relatively low in iodine on the basis of our present knowledge of the normal iodine content of the atmosphere. It therefore appears that after the iodine content of the solution is reduced to some point below 1 p. p. b., the iodine content of the air rather than the iodine content of the solution may become a limiting factor in the production of plant materials low in iodine.

Experiments with Micro-organisms

An initial experiment was conducted with representative bacterial species to find whether any of these species had iodine requirements large enough to be detected by a simple technique. Species of *Bacillus*, *Pseudomonas*, *Escherichia*, *Phytomonas*, and *Aerobacter* on repeated subculturing in iodine-free inverted-sucrose medium failed to exhibit any diminution of growth. Treatments with added iodine at rates of 0.01, 0.1, and 1 p. p. m. were tried as well as the iodine-free control. Subculturing was always from the iodine-free control culture. Although the calculated original iodine content of the medium amounted to only a few parts per billion, atmospheric contamination may have raised the iodine level somewhat since the preliminary trials were carried out in a bacteriological laboratory where the atmospheric iodine might be expected to be relatively high.

More intensive work was carried out on the bacterium *Azotobacter agile*.[8] Aside from the economic importance of the *Azotobacter* and reports of growth responses of *Azotobacter chroococcum* to iodine, the use of this organism permits the use of the more readily purified ethanol as an energy source and the elimination of nitrates from the medium.

This organism has been carried for a year by periodic subculturing in the iodine-free ethanol medium without a progressive reduction in growth rate or an apparent diminution of maximum turbidity of the cultures. The cultures were kept in a room believed to be uncontaminated by iodine. Since the medium is known to have had a maximal iodine concentration of the order of 0.001 p. p. b., it seems likely that iodine is not essential to the growth of *Azotobacter agile* in concentrations greater than 0.001 p. p. b.

Table 5.—*Stimulation of nitrogen fixation by Azotobacter agile in the presence of potassium iodide*

Iodine added (p. p. m.)	Total nitrogen fixed per flask after—			
	3 days	5 days	8 days	15 days
	Milligram	*Milligram*	*Milligram*	*Milligram*
0	3.52±0.29	12.8±1.0	25.3±0.9	26.1±0.9
0.01	4.71± .21	16.7± .7	24.7± .6	25.0± .5
0.1	5.33± .22	18.7± .8	27.1± .2	27.3± .2
1	5.76± .11	17.4± .4	26.9± .5	
50	4.94± .40	17.2± .2	25.1± .6	27.0± .4

Under certain conditions, a stimulation of growth of *Azotobacter agile* in the presence of added iodides was observed. In general, growth rate was stimulated while the maximum turbidity was little affected. The results of a typical experiment are presented in table 5. Apparently such results are due to the action of iodine on toxic

[8] The original culture of this organism was obtained by Dr. C. P. Hegarty from Dr. W. W. Umbreit of the University of Wisconsin.

constituents in the medium rather than to an essential nature of iodine in the nutrition of the organism.

Experiments now in progress indicate that iodides antidote the toxic action of copper on *Azotobacter agile*. Thus in the presence of 1 p. p. m. of copper in the substrate, maximum growth was achieved in the presence of 1 and 10 p. p. m. of iodine before any growth was apparent in the controls lacking added iodine, although visible growth was delayed 3 to 4 weeks in both cases. It has not been established, however, that all of the observed responses to iodine additions are due to the antidoting action on copper toxicity, and it is possible that other toxic factors are involved. In this connection, the work of Steinberg (*20*) is pertinent. He found that the toxicity of silver to *Aspergillus niger* is antagonized to a high degree by iodide, less so by bromide, and not at all by chloride. The solubilities of the silver halides appeared to be the important factor.

The growth of a mixed culture of algae in the iodine-free medium was also studied. Additions of iodine between the levels of 1 p. p. b. and 50 p. p. m. produced no apparent effect on growth.

DISCUSSION

The iodine requirements, if any, of most plants must be very low in view of the low normal iodine content of plants. The average iodine content of land plants usually lies in the range of 50 to 200 p. p. b. (*15*). The normal range of boron and manganese content is about 1,000 times this. Since 0.5 p. p. m. of these elements in solution cultures is sufficient for normal plant growth, it appears likely from analogy that an iodine concentration of the order of 1 p. p. b. would be adequate. Since nutrient solutions prepared from ordinary c. p. chemicals apparently have iodine concentrations of this order, the need for nutrient solution purification is obvious.

The presence of an iodine content in the normal range in the tomatoes grown in the specially prepared iodine-free solutions makes it impossible to state definitely on the basis of these experiments that iodine is not essential to the tomato. To show irrefutably that iodine is nonessential, plant material substantially lacking iodine must be grown.

It is possible that the iodine found in these plant tissues was derived from the atmosphere. In spite of the very low iodine content of the air, the observed accumulation of iodine would be no more striking than that of other substances. For example, if plants were able to absorb iodine from air containing 0.02 γ per cubic meter in the same relative proportion as they do carbon dioxide, this could account for an iodine contribution of the order of 100 p. p. b. More rigorous proof of the nonessential nature of iodine would therefore seem to require the use of air purification and unusual precautions against incidental contamination.

The distribution of iodine throughout the plant parts was found to be fairly uniform at both of the iodine levels investigated. Perusal of the literature reveals that, in general, fruits, starchy parts, roots, and seeds are lowest in iodine content, while green parts, especially leaves, are richest. It has frequently been suggested that iodine follows the transpirational stream in plant tissues.

The evidence seems clear that iodine is not essential to *Azotobacter agile*, although stimulations from added iodine may occur as a result of other effects on the environment. It seems possible that a similar relation may be found to hold for plants. The erratic nature and relatively small magnitude of the growth responses of plants to iodine, as well as the lack of specific deficiency symptoms, supports the view that iodine acts by ameliorating detrimental aspects of the environment. Here also should be mentioned the lack of evidence of widespread crop failure in extremely goiterous regions.

EFFECTS OF IODINE ADDITION TO SOILS

EXPERIMENTAL RESULTS

In accord with the second line of attack mentioned in the introduction, preliminary field-plot and greenhouse trials with iodine were conducted on a number of Oregon soils with various crops. Potassium iodide additions equivalent to 2 to 6 pounds of iodine per acre were applied following a complete fertilizer treatment. The iodine was applied in the form of a dilute solution before seeding in the case of annual plants, and during the early seasonal growth stages in the case of perennials.

While the results from some 40 such trials were largely negative, 2 soils were subjected to statistically interpretable tests. Replicated pot-culture trials were conducted with alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) on Aiken clay loam and Deschutes sandy loam. The Aiken soil had given indications of a response of alfalfa to iodine treatment.[9] The Deschutes soil came from Deschutes County, Oreg., where endemic goiter in human beings and farm animals has long been recognized. The soil itself was found to contain the low iodine content of 0.5 p. p. m.

The data for the first three cuttings on each trial are given in table 6. The yield data for further cuttings were erratic. Five and ten jars, respectively, were used per treatment in the alfalfa and red clover trials. Each soil received a complete fertilizer treatment before seeding.

Yield increases that seem to be statistically significant were obtained with the Aiken soil only, on the first and second cuttings. A slight response was found with the first cutting only on the Deschutes soil. It may be noted that for the first cuttings of red clover on both soils, and for the first cutting of alfalfa on the Aiken soil, the yield responses are proportionately greater when the green weights are considered; that is, "succulence" as well as total yield has been increased.

The crude protein content as well as the yield was increased in those cases in which iodine responses were obtained. This is of interest in view of Brenchley's (*3*) observation that nontoxic iodine dosages frequently increased the nitrogen content of barley and legumes.

[9] CARLSON, W. E. A CONTRIBUTION TO THE ROLE OF IODINE IN THE NUTRITION OF CERTAIN PLANTS. 83 pp. Corvallis, Oreg. (Thesis, Oreg. State Col.) Carlson found the closely related Olympic series to be the only one of four Oregon soils investigated that responded to potassium iodide additions in preliminary trials. The Aiken soil was taken from the same hillside as Carlson's Olympic soil. The samples were taken from a virgin profile where contamination with spray residues was out of the question.

TABLE 6.—*The effects of potassium iodide treatment on yields of alfalfa and red clover grown on Aiken clay loam and Deschutes sandy loam, 1937–38*

Item	Check	2 pounds iodine per acre	4 pounds iodine per acre
Aiken clay loam:			
Alfalfa, seeded June 1937, mean yields:			
First cutting, Sept. 27, 1937:			
Green weight, grams	7.60±0.45	10.40±0.45	10.80±0.45
Dry weight, do	2.85±0.22	3.65±0.30	3.75±0.15
Second cutting, Dec. 2, 1937:			
Dry weight, do	5.90±0.07	6.40±0.30	7.50±0.67
Third cutting, Mar. 17, 1938:			
Green weight, do	33.0±2.2	40.0±3.8	33.0±1.5
Dry weight, do	6.80±0.40	8.20±1.50	6.80±0.30
Iodine content (combined first and second cuttings (dry-weight basis)), parts per billion	384	415	361
Red clover, seeded October 1937, mean yields:			
First cutting, May 26, 1938:			
Green weight, grams	209±18	262±14	255±8
Dry weight, do	54.0±5.0	61.0±2.6	57.1±2.7
Second cutting [1]	(1)	(1)	(1)
Third cutting, Aug. 24, 1938:			
Green weight, do	112±4	105±3	113±3
Dry weight, do	29.3±1.3	28.3±1.0	29.6±2.1
Iodine content:			
First cutting, parts per billion	376	213	279
Third cutting, do	276	196	198
Protein content:			
First cutting, percent	15.55	16.89	17.63
Third cutting, do	15.24	14.85	14.85
Deschutes sandy loam:			
Alfalfa, seeded June 1937, mean yields:			
First cutting, Sept. 27, 1937:			
Green weight, grams	5.60±0.38	6.00±.022	6.20±0.60
Dry weight, do	1.95±0.15	2.10±0.22	2.10±0.22
Second cutting, Dec. 2, 1937:			
Dry weight, do	5.85±0.60	5.15±0.45	5.95±0.38
Third cutting Mar. 17, 1938:			
Green weight, do	39.00±1.5	34.0±3.8	32.0±3.8
Dry weight, do	8.40±0.30	7.45±0.67	6.90±0.60
Iodine content (combined first and second cuttings (dry-weight basis)), parts per billion	265	400	415
Red clover, seeded October 1937, mean yields:			
First cutting, May 26, 1938:			
Green weight, grams	191±10	215±7	206±5
Dry weight, do	46.6±3.3	47.7±1.0	45.9±1.1
Second cutting, July 15, 1938:			
Green weight, do	140±10	116±11	120±9
Dry weight, do	31.7±4.1	26.2±2.8	28.7±2.2
Third cutting, Aug. 24, 1938:			
Green weight, do	45.4±4.9	40.0±5.6	42.6±2.9
Dry weight, do	11.0±1.1	9.1±1.2	10.3±0.6
Iodine content:			
First cutting, parts per billion	186	492	712
Second cutting, do	277		612
Third cutting, do	132		486
Fourth cutting, do	189		484
Protein content:			
First cutting, percent	16.94	17.5	17.54
Second cutting, do	14.91	16.10	15.38

[1] Lost.

The plant iodine content was related to the iodine treatment in the case of the Deschutes soil which had a basal iodine content of 0.5 p. p. m., but not in the case of the Aiken soil which had the relatively high iodine content of 16.4 p. p. m. The iodine in the latter soil was largely present in the organic matter, as was shown by extraction with solvents that varied in their ability to extract humus. Thus, a water extraction yielded 1.4 p. p. m. or 7 percent of the total iodine in the soil; a 2-percent potassium carbonate extraction yielded 7.7 p. p. m. or 47 percent; while a 1-percent potassium hydroxide extraction yielded 12.3 p. p. m. or 75 percent. The water extract was almost colorless, the

potassium carbonate extract was cherry red, while the potassium hydroxide extract was very dark in color.

The difference in the ability of these two soils to retain iodine was also shown by the following leaching experiments. Small samples of soil which had been treated with 1 and 10 p. p. m. of added iodine were placed in duplicate in small Büchner funnels and leached for 2 months with small daily additions of distilled water. The recovery of total iodine from the Deschutes sandy loam was 16 and 46 percent for the soil samples containing 1 and 10 p. p. m., respectively, of added iodine; for the Aiken clay loam the recovery was 0.6, 1.7, and 0.6 percent for the samples containing 0, 1, and 10 p. p. m., respectively, of added iodine.

In accord with the results of other workers, early nontoxic applications of iodine to soils did not give the striking increases in plant iodine content that are noted in solution culture work. The iodine content values obtained are in agreement with those summarized by McClendon (*15*). Incidentally, natural waters were low in iodine, although a few deep wells were found to contain as much as 6 to 120 p. p. b.

DISCUSSION

The results of additions of iodine to Oregon soils have been largely negative. Well-replicated pot-culture trials with legumes on Aiken clay loam have given a small response to iodine treatment that seems to be statistically significant. The few other cases where stimulative actions have been found in preliminary trials must be tested more thoroughly before definite statements can be made.

The largely negative results of iodine fertilization of soils in a region of endemic goiter support the conclusion, based on solution culture trials, that iodine is either not essential in plant nutrition, or is required in very minute amounts only. It is concluded that bona fide responses to iodine treatment are probably due to antagonistic actions in view of the small magnitude and erratic occurrence of soil responses to iodine treatments found by the writers and by other workers.

The stimulation of legume yields on Aiken clay loam is particularly interesting in this connection. The fact that a positive growth response was obtained with low iodine applications on a soil containing a relatively high iodine content, although the iodine content of the plant material was not affected by the iodine treatment; that the response was most apparent on the first cutting and was found only on the first and second cuttings; that the response was greater on the green-weight than on the dry-weight basis; and that the nitrogen content was increased when yield responses were obtained, all point to an indirect effect of the iodine, rather than to the remedying of an iodine deficiency.

The magnitude of the iodine response on the Aiken soil and the evidence for responses on other soils are not great enough to warrant hopes of economic returns from iodine fertilization.

SUMMARY

The effects produced by the addition of small amounts of potassium iodide to nutrient solutions prepared from c. p. chemicals and dis-

tilled water have been investigated. Significant increases in yield were not obtained with corn, barley, or lettuce grown in these solutions. The iodine content of the plant material was found to be linearly related to the concentration of added iodine in the solution cultures. On the basis of these data and certain assumptions, the maximal iodine content of the basal nutrient solutions was calculated to be of the order of 1 p. p. b.

Methods were developed for reducing the iodine content of certain nutrient chemicals to less than 1 p. p. b. Using such chemicals and redistilled water, the writers prepared nutrient solutions having an iodine content of the order of 0.001 p. p. b. Tomatoes failed to give growth responses to iodine additions in these solutions.

Azotobacter agile was subcultured at periodic intervals for approximately 1 year in a 1.1 percent ethanol medium having an iodine content of the order of 0.001 p. p. b. without a reduction in the normal growth rate.

Exploratory pot-culture and field-plot trials on a number of Oregon soils with early nontoxic iodine additions have been largely negative. A statistically interpretable greenhouse trial on Aiken clay loam showed small responses with alfalfa and red clover on the initial cuttings. Iodine may act indirectly rather than by remedying a deficiency of an essential element.

LITERATURE CITED

(1) Branham, Sara E.
1929. The effects of certain chemical compounds upon the course of gas production by baker's yeast. Jour. Bact. 18: 247–264, illus.

(2) Bratton, A. C., and McClendon, J. F.
1938. Determination of iodine in drinking water, urine, and substances containing only about 1000 times as much organic matter as iodine. Indus. and Engin. Chem., Analyt. Ed. 10: 600–605.

(3) Brenchley, Winifred E.
1924. The effect of iodine on soils and plants. Ann. Appl. Biol. 11: 86–111, illus.

(4) Cotton, Marjorie.
1930. Toxic effects of iodine and nickel on buckwheat grown in solutions cultures. Torrey Bot. Club Bul. 57: 127–140, illus.

(5) Davenport, C. B., and Love, Albert G.
1920. Defects found in drafted men. Sci. Monthly 10: [5]–25, 125–141, illus.

(6) Greaves, J. E.
1933. Some factors influencing nitrogen fixation. Soil Sci. 36: 267–280.

(7) ——— Zobell, C. E., and Greaves, J. Dudley.
1928. The influence of iodine upon the growth and metabolism of yeasts. Jour. Bact. 16: 409–430.

(8) Harvey, C. O.
1935. The determination of iodine in biological substances. 43 pp., illus. London. (Med. Res. Council, Spec. Rpt. Ser. No. 201.)

(9) Ishikawa, Mitsuteru.
1928. Influence of iodide on bacterial decomposition of nitrogenous substances. Jour. Infect. Dis. 43: [321]–326.

(10) Itano, A., and Matsuura, A.
1933. Influence of iodine on physiological activities of microorganisms. Ōhara Inst. f. Landw. Forsch. Ber. 6: 73–81.

(11) KOLNITZ, HARRY VON, and REMINGTON, ROE E.
1933. A SIMPLIFIED KARNS TECHNIC FOR THE MICRO-ESTIMATION OF IODINE. Indus. and Engin. Chem., Analyt. Ed. 5: 38–39, illus.
(12) LEWIS, J. C., and POWERS, W. L.
1941. ANTAGONISTIC ACTION OF CHLORIDES ON THE TOXICITY OF IODIDES TO CORN. Plant Physiol. 16: 393–398.
(13) MACK, WARREN B., and BRASHER, EUGENE P.
1936. THE INFLUENCE OF COMMERCIAL FERTILIZERS, POTASSIUM IODIDE, AND SOIL ACIDITY ON THE IODINE CONTENT OF CERTAIN VEGETABLES. Jour. Agr. Res. 53: 789–800, illus.
(14) MAZÉ, P.
1919. RECHERCHE D'UNE SOLUTION PUREMENT MINÉRALE CAPABLE D'ASSURER L'ÉVOLUTION COMPLÈTE DU MAIS CULTIVÉ À L'ABRI DES MICROBES. Inst. Pasteur [Paris] Ann. 33: [139]–173, illus.
(15) MCCLENDON, J. F.
1939 IODINE AND THE INCIDENCE OF GOITER. 126 pp., illus. Minneapolis and London.
(16) ORR, J. B., KELLY, F. C., and STUART, G. L.
1928. THE EFFECT OF IODINE MANURING ON THE IODINE CONTENT OF PLANTS. Jour. Agr. Sci. [England] 18: 159–161.
(17) ORR, J. B., and LEITCH, I.
1929. IODINE IN NUTRITION, A REVIEW OF EXISTING INFORMATION. 108 pp., illus. London. (Med. Res. Council, Spec. Rpt. Ser. No. 123.)
(18) REITH, JANS FRANS, and DIJK, CAROLUS PAULUS VAN.
1937. INVESTIGATIONS ON THE DETERMINATION OF MICRO-QUANTITIES OF IODINE. Biochem. Jour. 31: 2128–2135, illus.
(19) SCHARRER, K., and SCHROPP, W.
1937. WEITERE GEFÄSS UND WASSERKULTURVERSUCHE ÜBER DIE WIRKUNG DES BORS ALLEIN UND IN KOMBINATION MIT JOD IN DÜNGEMITTELN. Phytopath. Ztschr. 10: [57]–78, illus.
(20) STEINBERG, ROBERT A.
1935. NUTRIENT-SOLUTION PURIFICATION FOR REMOVAL OF HEAVY METALS IN DEFICIENCY INVESTIGATIONS WITH ASPERGILLUS NIGER. Jour. Agr. Res. 51: 413–424.
(21) STOKLASA, JULIUS.
1926. DIE PHYSIOLOGISCHE FUNKTION DES JODS BEIM BAU UND BETRIEBSSTOFFWECHSEL IN DER CHLOROPHYLLHALTIGEN UND CHOROPHYLLOSEN ZELLE. Biochem. Ztschr. 176: [38]–51.
(22) ———
1929. ÜBER DEN EINFLUSS DES JODIONS AUF DAS WACHSTUM UND DIE ZELLVERMEHRUNG DER HALOPHYTEN. Biochem. Ztschr. 211: [213]–228, illus.
(23) WHITE, PHILIP R.
1938. ACCESSORY SALTS IN THE NUTRITION OF EXCISED TOMATO ROOTS. Plant Physiol. 13: 391–389, illus.

MEETING THE VITAMIN D REQUIREMENT OF PIGS WITH ALFALFA HAY AND WINTER SUNSHINE [1]

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INTRODUCTION

It has been shown by the authors (*3*)[2] that pigs require vitamin D even when receiving rations higher in calcium and phosphorus than are ordinarily recommended. Meeting this requirement is of practical importance and presents a problem under the usual winter-feeding conditions in Minnesota, where rather close confinement of pigs is necessary during the winter months.

It is not generally understood that all common pig feeds, especially grains and protein supplements, are completely lacking in vitamin D. Because of the importance of this vitamin in the nutrition of pigs, further experiments were conducted to determine how it may be provided and also to find the extent of the pig's need for it.

MATERIALS AND METHODS

In addition to feed consumption, weight records, and the gross symptoms of the pigs, the level of calcium in the blood plasma, from single blood samples obtained from tail bleeding, was used to determine the need of the pigs for vitamin D. Since alfalfa hay may contain vitamin D (*6*), and is one of the few roughages used in pig rations, it was fed in each of the three experiments in this investigation. In the first two experiments three lots of hay were used, lot I in the first experiment and lots II and III in the second. At the conclusion of these two experiments the vitamin D content of the three lots of hay was determined. A fourth lot of hay for use in a third experiment was also assayed. This hay was cured by exposure to a number of hours of sunlight approximating that given to hay in the usual manner of curing on farms. For this purpose a small field of alfalfa was mowed at 10:00 a. m. and the hay was put in storage the next day at approximately 1:00 p. m. During the entire curing period the weather was clear, thus permitting the maximum amount of exposure.

Quantities of these four lots of hay were assayed for vitamin D according to the United States Pharmacopoeia XI procedure by which it was possible to determine the approximate number of International Units of vitamin D in each lot of hay. Rachitogenic diet No. 3143 of McCollum et al. (*4*) was employed. The vitamin D content of the four lots of hay, as shown in table 1, was 0.15, 0.39, 1.46, and 0.85 International Units per gram. It should be noted that Wallis (*7*) reports 10.45 International Units for alfalfa leaves and 1.72 for

[1] Received for publication April 2, 1941. Paper No. 1898, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 648.

alfalfa stems. The discrepancy between his results and those here shown is apparently due to a difference in healing obtained with the reference oil.

TABLE 1.—*Results of vitamin D assays with rats of the different lots of alfalfa hay fed in the pig experiments*

Material, level of feeding, and quantity consumed during the first 8 days of the assay period	Rats used	Average healing (line test)[1]	Vitamin D per gram (calculated)[2]
	Number		*International Units*
Standard reference oil, 0.4 International Unit daily	10	+2.5	
Lot I alfalfa, 20 percent of diet, 1.47 gm. average daily intake	12	+1.4	0.15
Lot II alfalfa, 10 percent of diet, 0.69 gm. average daily intake	13	+1.7	.39
Lot III alfalfa, 5 percent of diet, 0.34 gm. average daily intake	9	+3.1	1.46
Lot IV alfalfa, 10 percent of diet, 0.66 gm. average daily intake	15	+3.5	.85

[1] The numerical degrees of healing suggested by Bills et al. *(1)* were followed in interpreting the line tests.
[2] The calculated values are not strictly correct since they were made by simple proportion, as is customary. It is known that the relationship between the units of vitamin D received and the degree of healing is curvilineal, and that vitamin D units in an unknown may be calculated by arithmetical proportion only when the unknown and the standard reference will give the same degree of healing.

EXPERIMENT 1

PROCEDURE

In experiment 1, begun December 12, 1935, three lots of six pigs each were used. In lot 1 there were one Duroc-Jersey, one Poland China, and four Hampshires. In each of the other two lots there were two Poland Chinas and four Duroc-Jerseys. The pigs were started on experiment shortly after weaning and were fed individually twice daily. All lots in this experiment, as well as in the experiments which follow, were housed in a hog barn and were not allowed access to the outside except as called for by the experimental procedure.

The ration fed lot 1 consisted of yellow corn, 86.84 percent; dry-rendered tankage, 11.80 percent; sodium chloride, 0.50 percent; bonemeal, 0.26 percent; limestone, 0.45 percent; and unirradiated yeast, 0.15 percent. This ration provided 0.65 percent of phosphorus and 1.07 percent of calcium. The pigs receiving it were allowed to become rachitic and were then used to test the antirachitic potency of alfalfa. Lot 2 received the above-described ration and had access to the outside at all times. This plan was followed to determine whether pigs so handled would receive enough benefit from winter sunshine to preclude the necessity of adding a vitamin D supplement to their ration. A daily record was kept of the hours of sunshine and the approximate amount of time the pigs spent in their outside lot from the beginning of the experiment until its close. The only change made in the above ration for lot 3 was the substitution of irradiated yeast for unirradiated yeast; thus this ration served as the control since a ration containing 0.15 percent of irradiated yeast had been found by the authors *(3)* to afford complete protection from rickets.

RESULTS

As the body stores of vitamin D became depleted the average percent of calcium in 100 cc. of blood plasma of five of the pigs in lot 1 decreased from 10.47 mg. on January 9 to 6.51 mg. on February 24.

One week later the remaining pig was bled and showed 6.91 mg. All of these pigs showed marked reduction in feed intake and negligible growth or a loss in weight, and two pigs exhibited calcium-deficiency tetany. That these symptoms were the result of a vitamin D deficiency was shown by the fact that the plasma calcium of one of the pigs manifesting tetany increased from 5.58 mg. to 11.05 mg. in 12 days after the pig had received 4 ounces of U. S. P. cod-liver oil. Furthermore, the pigs in lot 3, the control lot, showed no reduction in plasma calcium and their appetites and growth were excellent.

Four of the pigs in lot 1 were given lot I alfalfa as 1 percent of their ration and the remaining pig, one of the two manifesting calcium-deficiency tetany, was given the alfalfa as 5 percent of its ration. However, in order to save the life of this pig 8 ounces of cod-liver oil were administered over a period of 4 days, after which it recovered. After 16 feedings of the ration containing 1 percent of lot I alfalfa, the plasma calcium of each of the pigs was lower than before it was given. Consequently the alfalfa was increased to 5 percent. After 12 feedings of this ration one of the four pigs had a convulsion; it was given 4 ounces of cod-liver oil and removed from the experiment. The remaining three pigs received 16 feedings of this ration but showed no improvement. The alfalfa was then increased to 10 percent of the ration. The lack of recovery from 21 feedings of this ration is not surprising in view of the small amount of vitamin D in this hay (table 1). During this period of the experiment the three pigs, averaging approximately 200 pounds, ate on an average 4.85 pounds of feed per day, and each therefore received but 33 International Units of vitamin D daily.

The pigs in lot 2 received the same ration as those in lot 1, but in spite of having access to an outside lot, they needed more vitamin D during the early part of the experiment than they obtained from exposure to sunlight. This was indicated by the incidence of stiffness and slight reductions of plasma calcium in several of the pigs and by slower average growth for the lot as compared with lot 3, the pigs in the latter lot averaging 7.3 pounds per pig heavier than those in lot 2. This difference, however, developed during the first part of the experiment; during the latter part, February 6 to March 20, lot 2 gained equally as well as lot 3. It is interesting to note that the improvement in growth of the pigs of lot 2 coincided with the period of greater sunshine during which, unless the weather was severe, they spent more time outside.

EXPERIMENT 2

PROCEDURE

In order to make a further study of the value of winter sunshine in curing rickets and of alfalfa hay in preventing rickets two lots of six pigs each were started on experiment December 21, 1936. The pigs, three Duroc-Jerseys and three Poland Chinas in each lot, averaged 45 pounds in weight. The ration fed to lot 1 for the purpose of testing the value of winter sunshine, consisted of corn, 86 percent; dry-rendered tankage, 12 percent; sodium chloride, 0.50 percent; bonemeal, 1.0 percent; and limestone, 0.50 percent. The calcium and phosphorus in the ration were 1.05 percent and 0.63 percent, respectively. Lot 2 received 95 parts of this ration and 5 parts of lot II alfalfa meal.

The inclusion of the alfalfa did not appreciably change the percentages of calcium and phosphorus in this ration as compared with that for lot 1. The ration for each lot was self-fed. Individual weights of the pigs and the feed eaten by each lot were recorded at 2-week intervals.

RESULTS

The data in table 2 show that in 8 weeks there was a reduction in the average plasma calcium values of the pigs in lot 1 from 10.95 mg. to 6.89 mg. Some of these pigs were lame, but lameness was not characteristic of all the pigs in this lot. Since it was clearly evident from the blood picture that these pigs were rachitic, they were exposed to the sunlight for 2 weeks, beginning February 16. The exposure was limited to the period from 10:30 a. m. to 3:30 p. m. Because of cloudy weather they were exposed for an average of only 1.48 hours per day. This treatment caused the plasma calcium to increase to 10.52 mg. (table 2), and this increase was accompanied by a marked improvement in appetite, growth, and appearance. Solar irradiation was continued for another 2 weeks, which resulted in further improvement, although sufficient irradiation may have been given during the first period to account for the continued improvement.

TABLE 2.—*Calcium in 100 cc. of blood plasma of pigs as affected by exposure to sunshine and by dietary supplements during experiment 2, 1936–37*

Calcium in 100 cc. of plasma in lot 1 [1]					Calcium in 100 cc. of plasma in lot 2 [2]				
Pig No.[3] and breed	Dec. 21	Feb. 15	Mar. 1	Mar. 15	Pig No.[3] and breed	Dec. 21	Feb. 15	Mar. 1	Mar. 15
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1–DJ	10.05	6.96	10.64	11.56	7–DJ	11.01	10.23	11.26	11.26
2–DJ	10.95	6.65	10.69	10.69	8–DJ	10.37	9.31	9.06	11.15
3–DJ	10.69	6.91	9.52	11.56	9–DJ	11.00	8.70	9.82	10.95
4–PC	11.64	7.42	10.49	11.51	10–PC	10.69	10.13	10.54	10.74
5–PC	11.01	7.37	10.74	11.72	11–PC	10.42	9.62	10.03	10.95
6–PC	11.33	6.04	11.05	11.36	12–PC	10.95	10.18	10.74	10.95
Mean	10.95	6.89	10.52	11.40	Mean	10.74	9.70	10.24	11.00

[1] Exposed to sunlight beginning Feb. 16.
[2] Ration included 5 percent of alfalfa meal. For 2 weeks prior to Feb. 15 these pigs received an average of 38 I. U. of vitamin D per animal per day; from Feb. 15 to Mar. 1 they received 183; and from Mar. 1 to Mar. 15, 241.
[3] DJ and PC signify Duroc-Jersey and Poland China, respectively.

The blood picture of lot 2 was much more favorable than that of lot 1. From December 21 to February 15 there was an average reduction of only 1.04 mg. in the plasma calcium. Although there was a variation among the pigs in this respect the plasma calcium of each showed some reduction. It was evident that the lot II alfalfa hay furnished enough vitamin D to give some protection from rickets. On February 15 the pigs averaged 102 pounds in weight and for the 2 weeks prior to that date had received an average of 38 International Units of vitamin D per pig per day. On February 15 the supply of this lot of hay was exhausted and another lot (lot III) was fed. That this hay contained enough vitamin D for the pigs was evident from the increase, with the exception of pig 8, in their plasma calcium values in 2 weeks and a further increase for all the pigs in another 2 weeks. From February 15 to March 1 the pigs received 183 International

Units of vitamin D daily per pig, and from March 1 to March 15 they received 241 International Units. In the writers first investigation (*3*) it was found that irradiated yeast as 0.10 percent of the ration failed to prevent rickets but as 0.15 percent it afforded complete protection. Although this yeast was not assayed, according to the manufacturer it probably contained 900 International Units per gram. Thus, approximately 1,700 International Units of vitamin D per pig per day were required. This discrepancy between the amount of vitamin D required by pigs when supplied in irradiated yeast and when supplied in alfalfa hay requires further study.

The difference in the appetite of pigs in lot 1 and lot 2 and the improvement of both in this respect after receiving what was apparently an adequate amount of vitamin D (table 3) illustrates the importance of meeting the need of pigs for this vitamin. This is true even of lot 2 in which only a slight deficiency was encountered as judged by the small reductions of blood-plasma calcium and by the total absence of visible symptoms of rickets. As expected, the increase in feed consumption was accompanied by an improvement in growth. Lot 1 gained 54 percent faster during the 2 weeks after exposure to the winter sunshine, and lot 2 gained 43 percent faster during the 2 weeks after the change from lot II to lot III alfalfa, than during the 2 weeks preceding these changes.

TABLE 3.—*Average daily feed consumed per pig at different times during experiment 2 1936–37*

Lot No. [1]	Dec. 21–Jan. 4	Jan. 4–Jan. 18	Jan. 18–Feb. 1	Feb. 1–Feb. 15	Feb. 15–Mar. 1	Mar. 1–Mar. 15
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	2.61	3.05	3.24	3.23	4.48	6.21
2	2.56	3.16	3.72	4.28	5.52	7.29

[1] See footnotes 1 and 2, table 2.

EXPERIMENT 3

PROCEDURE

In order to obtain more information on the requirement of the pig for vitamin D, a third experiment was initiated November 15, 1937. Six lots of pigs, averaging 47 pounds in weight, were used for studying (1) the value of winter sunshine and of alfalfa containing 0.85 of an International Unit of vitamin D per gram (lot IV) in curing rickets in pigs, (2) the value of this same alfalfa in preventing rickets, and (3) the effect of the concentration of calcium and phosphorus in the ration on the vitamin D requirement. Each lot consisted of one Duroc-Jersey, two Poland Chinas, and three Hampshires. However, during the course of the experiment one pig was removed from lot 1 and one from lot 3 for failure to eat their food. Records for these pigs have been omitted from the report of this experiment.

The percentages of calcium and of phosphorus in rations of swine vary widely, depending upon the protein supplement used. During summer feeding, when providing vitamin D is not a problem, similar results can be obtained from protein supplements consisting largely of soybean-oil meal or dry-rendered tankage or of meat and bone scraps. For young pigs, when the demand for protein is high, the

phosphorus content of such rations is approximately 0.40 percent, 0.60 percent, and 1.2 percent, respectively. Since it was desired to use rations typical of those used in practice, concentrations of phosphorus similar to the above were employed. These rations, shown in table 4, which were self-fed to lots 1, 2, and 3, had a phosphorus content of 0.42 percent, 0.64 percent, and 1.19 percent, respectively. The calcium-phosphorus ratios were 1.5 for rations 1 and 2 and 1.6 for ration 3. Excepting ration 1, the calcium levels were typical of those in swine rations employing the protein supplements mentioned above. Additional calcium was added to ration 1 in order that the calcium-phosphorus ratios in the three rations might be similar.

TABLE 4.—*Rations used in experiment 3, 1937–38*

Ration 1		Ration 2		Ration 3	
Component	Proportion	Component	Proportion	Component	Proportion
	Percent		*Percent*		*Percent*
Yellow corn	76.66	Yellow corn	75.13	Yellow corn	70.50
Soybean-oil meal (80 percent), tankage (20 percent).	22.00	Soybean-oil meal (80 percent), tankage (20 percent).	22.30	Soybean-oil meal (80 percent), tankage (20 percent).	23.25
Sodium chloride	.50	Sodium chloride	.50	Sodium chloride	.50
Limestone	.84	Limestone	.47	Limestone	.25
		Bonemeal	1.60	Bonemeal	5.50

Pigs of lots 4 and 5 received the same rations as lots 1 and 2 except that 5 percent of the ration consisted of lot IV alfalfa hay containing 0.85 of an International Unit of vitamin D per gram. This hay was cured under ideal conditions. The pigs of lots 4 and 5 were confined indoors. It should be recalled that in experiment 1 pigs fed a ration containing no vitamin D, but given voluntary access to an outside runway, did not receive sufficient exposure to sunlight during December and January; that in experiment 2 rickets was cured when pigs were given exposure to winter sunshine; and that lot III alfalfa, as 5 percent of the ration, contained enough vitamin D (1.46 International Units per gram) to meet the pigs' requirement for this vitamin. It was, therefore, considered desirable to feed the pigs of lot 6, in addition to ration 1, lot IV alfalfa hay, and also to allow them voluntary access to an outside runway. Since alfalfa hay is commonly self-fed to hogs on farms, this method of feeding the hay was followed with this lot. Feed and weight records for the three lots were taken at biweekly intervals.

RESULTS

The calcium analyses of the blood plasma and the feed intake of the pigs in experiment 3 are shown in tables 5 and 6, respectively. As was expected, the calcium content of the blood plasma of representative pigs was normal 1 week after the beginning of the experiment. After 8 weeks, on January 11, all of the pigs in lot 1 except No. 1 and all in lot 2 except No. 7 showed plasma calcium concentration considerably below normal. The two exceptions, litter mates, were removed from their respective lots on January 13 and given ration 1, the low-calcium, low-phosphorus ration which pig No. 1 had been receiving. Not until February 7, 12 weeks after the start

of the experiment, was there a reduction in the plasma calcium of these two pigs; namely, to 9.65 mg. for No. 1 and 9.14 mg. for No. 7. These pigs, subjected to the same preexperimental treatment as the others, presented such a marked contrast to the other 9 pigs in lots 1 and 2 that there would appear to be important hereditary differences among pigs in their requirement for vitamin D.

One pig in lot 3, No. 18, receiving ration 3, and one in lot 4, No. 22, receiving ration 1 supplemented with lot IV alfalfa, also showed slight reductions in plasma calcium values at the end of 8 weeks. However, when calcium analyses were next made No. 22 had a plasma calcium value of 10.33 mg. on February 4 and No. 18 one of 11.16 mg. on February 7.

It is interesting to note that the higher calcium and phosphorus rations fed to lots 2 and 3 as compared with lot 1 apparently reduced the rate of utilization of the vitamin D stores for the 8 weeks, because the average plasma calcium value for lot 2 was 1.5 mg. higher than for lot 1, and none of the pigs in lot 3 showed an appreciable reduction in this value except No. 18, as has been mentioned.

TABLE 5.—*Calcium in 100 cc. of blood plasma of pigs as affected by exposure to sunshine and by dietary supplements during experiment 3, 1937–38*

Lot No.	Pig No. and breed [1]	Calcium in 100 cc. of plasma on—							
		Nov. 23	Jan. 11	Jan. 28	Feb. 4	Feb. 7	Feb. 8	Feb. 21	Feb. 28
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	1–DJ	10.31	10.57			9.65		8.94	
	2–PC	10.25	6.65	6.18			5.97		
1 [2]	3–PC		6.70	7.99			7.73	5.87	
	5–H		5.76	6.12			7.27		
	6–H	11.39	7.94	8.20			8.72	8.21	
	7–DJ	10.31	11.26			9.14		7.23	
	8–PC	10.79	7.54	4.57					
2 [2]	9–PC		9.33	11.21					
	10–H		8.19	10.69					
	11–H	11.06	9.87	12.27					
	12–H	6.40	11.26						
	13–DJ	11.33	10.77			11.26			10.14
	14–PC	11.50	12.11			11.37			10.29
3 [2]	16–H		11.36			12.20			9.15
	17–H	11.17	11.76			11.11			10.14
	18–H		9.53			11.16			9.72
	19–DJ	10.52	10.82		11.21				
	20–PC	11.12	11.01		11.32				
4 [3]	21–PC		11.91		12.20				
	22–H		9.92		10.33				
	23–H	10.74	10.97		11.11				
	24–H		10.72		10.69				
	25–DJ	10.63	11.31		11.68				
	26–PC		10.72		11.06				
5 [3]	27–PC	10.90	10.57		11.16				
	28–H		10.62		11.21				
	29–H	11.50	11.41		11.26				
	30–H		11.11		11.16				
	31–DJ	10.04	10.92		10.23				
	32–PC		11.71		11.21				
6 [4]	33–PC	10.52	10.42		11.16				
	34–H		10.97		11.11				
	35–H	11.77	10.87		10.48				
	36–H		11.26		11.83				

[1] DJ, PC, and H signify Duroc-Jersey, Poland China, and Hampshire, respectively.
[2] Received basic diet only, but lots 2 and 3 received more calcium and phosphorus than lot 1. The pigs in lot 2 were exposed to sunlight, beginning Jan. 13.
[3] Ration included 5 percent of alfalfa hay; pigs confined indoors.
[4] Ration included 5 percent of alfalfa hay; pigs had access to an outside runway.

TABLE 6.—*Average daily feed consumed per pig at different times during experiment 3, 1937–38*

Lot No.[1]	Nov. 16–Nov. 30	Nov. 30–Dec. 14	Dec. 14–Dec. 28	Dec. 28–Jan. 11	Jan. 11–Jan. 25	Jan. 25–Feb. 8	Feb. 8–Feb. 22	Feb. 22–Mar. 1
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1[2]	3.08	3.13	3.79	4.02	3.41	4.72	----------	----------
2[3]	2.71	3.12	3.70	4.15	3.64	4.77	----------	----------
3	2.73	2.68	2.81	3.56	4.27	5.98	5.03	5.97
4	2.65	3.19	3.45	4.24	4.40	4.12	----------	----------
5	2.80	2.96	2.91	4.02	3.95	3.69	----------	----------
6	2.87	3.34	3.62	4.37	4.57	4.03	----------	----------

[1] See Footnotes 1, 3, and 4, table 5.
[2] Lot 1 continued until Mar. 1, but feed records are not given for additional periods because of changes in ration.
[3] Lot 2 stopped Feb. 1.

Since pigs 2, 3, 5, and 6 on ration 1 were rachitic, it was decided to feed them the alfalfa that lots 4, 5, and 6 had been receiving. Accordingly, the hay was self-fed beginning January 13, and a record was kept of its consumption. By January 28 there was a slight increase in the plasma calcium of three of these pigs and a reduction in that of the fourth. By February 8, 26 days after the feeding of the hay had started, during which time they ate a quantity of hay consisting of 6.9 percent of their ration, the plasma calcium values of Nos. 5 and 6 were still higher, indicating some benefit from the vitamin D in the hay. However, by February 21 the calcium values for these pigs were again lower. Pigs 3 and 6 were continued on this ration until the close of the experiment on March 1 without any noticeable improvement in the rachitic condition.

The fact that lot 3 receiving ration 3, high in calcium and phosphorus, showed no need for vitamin D at the time the attempt to bring about recovery in lot 1 by means of alfalfa hay was meeting with failure, seemed to indicate that pigs require little if any vitamin D when receiving sufficiently high concentrations of calcium and phosphorus in the rations, and that, therefore, it should be possible to cure rickets by feeding such a ration. Accordingly, on February 8, pigs 2 and 5 were given ration 3. This did not improve their condition, which instead, became progressively worse; No. 2 had to be removed from the experiment 1 week later and by March 1 No. 5 had lost 6 pounds. Since pigs 1 and 7 had become slightly rachitic, as shown by reductions of blood-plasma calcium to 9.65 mg. and 9.14 mg., respectively, they too were changed to the high mineral ration on February 8, but the change was without benefit as shown by further reductions of blood-plasma calcium on February 21 to 8.94 mg. and 7.23 mg., respectively. Pigs 3 and 6 were changed to this ration on February 21 and continued on it until the close of the experiment on March 1, but the change was likewise without benefit.

In order to test the value of winter sunshine for curing rickets, the five pigs in lot 2 were given solar irradiation, beginning on January 13. For 14 days they received, on an average, approximately 45 minutes of irradiation per day. The remarkable benefit of this treatment is shown by the increase to normal in plasma calcium values on January 28. The stiffness exhibited by several of the pigs also disappeared.

The use of the high calcium and phosphorus ration 3 for lot 3 was distinctly beneficial in reducing the rate of the utilization of the body

stores of vitamin D. It was not until February 28 that there was any evidence from the plasma calcium values of a need for this vitamin. At that time there was a reduction of plasma calcium in each pig and an average reduction of 1.53 mg. for the lot as compared with the values on February 7. It is believed that if these pigs had been continued on experiment they would eventually have become as rachitic as the pigs in lots 2 and 3. From the standpoint of practical feeding it would be more costly to provide rations as high in calcium and phosphorus as ration 3 in order to obviate the need for added vitamin D during the months of little sunshine and confinement than to use a vitamin D supplement. This is suggested by the finding that the vitamin D requirement of pigs can be met by forced exposure to winter sunshine and by the results with lots 4, 5, and 6, to be discussed later, and also by the observation that lot 3 tried to avoid eating the bonemeal in the ration by allowing it to settle to the bottom of the feeder. Bohstedt (*2*) has also found that rations unusually high in minerals are not conducive to the best results with pigs.

It may be seen from the data in table 5 that lots 4 and 5, which received the same rations as lots 1 and 2 except that 5 percent of alfalfa containing 0.85 of an International Unit of vitamin D per gram was included, still showed normal plasma calcium values on February 4, 3½ weeks after lots 1 and 2 were severely rachitic. The pigs in lot 6 also showed normal plasma calcium values at that time. Thsee results could be expected in view of the fact that the average hay consumption of these pigs was 5.05 percent of their ration and from the further fact that they spent sufficient time in their outside runway to receive some exposure to sunshine. In order to facilitate the more extensive studies with lots 1 and 2 the last three lots of the experiment were discontinued on February 5.

It is apparent from this experiment that pigs require more vitamin D to recover from severe rickets than is required to protect them from the disease, even when their body stores of the vitamin are partially depleted. It is, therefore, particularly noteworthy that there was such a rapid recovery of the pigs in lot 2 as a result of exposure to January sunshine. This finding, agreeing with that of Sloan (*5*) for poultry in the vicinity of Ithaca, N. Y., is of practical importance in winter swine feeding, particularly in view of the generally accepted belief that winter sunshine is of no value in generating vitamin D in livestock.

SUMMARY AND CONCLUSION

The growing pig's requirement for vitamin D was investigated with fall-farrowed pigs of the Duroc-Jersey, Hampshire, and Poland China breeds in 3 different years. In some of the tests the pigs were allowed to become rachitic, as judged by reductions in blood-plasma calcium, and were then exposed to winter sunshine or were given alfalfa hay. In other tests attempts to provide the needed vitamin D were made at the beginning of the experimental period by confining the pigs indoors and feeding alfalfa as the source of the vitamin or by allowing them voluntary access to an outside runway with or without alfalfa hay. Four lots of alfalfa hay, all of which were assayed for vitamin D by the U. S. P. XI procedure, were used. The effect of low, medium, and high concentrations of calcium and of phosphorus on the vitamin D requirement was also studied.

It was found that rickets was cured in pigs exposed to January sunshine for an average of 45 minutes per pig per day for 2 weeks; no tests with shorter exposures were made. Similar results were obtained in another experiment with pigs exposed to February sunshine. Pigs fed indoors and allowed voluntary access to an outside runway did not receive sufficient solar irradiation during December and January. When alfalfa, containing 0.39 of an International Unit of vitamin D per gram was fed as 5 percent of the ration the pigs became rachitic. This condition was cured by using another alfalfa hay containing 1.46 International Units per gram as 5 percent of the ration. This hay provided approximately 200 to 250 International Units of vitamin D daily per pig. Pigs receiving an alfalfa hay containing 0.85 of an International Unit per gram, as 5 percent of the ration. did not become rachitic, although this hay failed to cure rickets at this percentage of the ration. Pigs allowed access to an outside runway and self-fed this alfalfa ate 5.05 percent of their ration as hay and did not become rachitic. This lot of hay was cured under ideal conditions, having been mowed in the forenoon and put in storage the afternoon of the next day during which time clear weather prevailed. The need for vitamin D was found to be inversely proportional to the calcium and phosphorus content of the ration, at the levels fed in these experiments.

The effectiveness of meeting the pig's requirement for vitamin D by feeding alfalfa hay and by allowing access to winter sunshine would depend, of course, upon the extent to which the hay had been exposed to sunshine during the curing process and upon the amount of sunshine and the severity of the weather during the winter months.

LITERATURE CITED

(1) Bills, Charles E., Honeywell, Edna M., Wirick, Alice M., and Nussmeier, Mildred.
1931. A critique of the line test for vitamin D. Jour. Biol. Chem. 90: 619–636, illus.

(2) Bohstedt, G.
1939. The mineral requirements of pigs. Amer. Soc. Anim. Prod. Proc. 32: 137–144.

(3) Johnson, D. W., and Palmer, L. S.
1939. Individual and breed variations in pigs on rations devoid of vitamin D. Jour. Agr. Res. 58: 929–939.

(4) McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A.
1922. A delicate biological test for calcium-depositing substances. Jour. Biol. Chem. 51: 41–49, illus.

(5) Sloan, H. J.
1934. The seasonal variation in the antirachitic effectiveness of sunshine. Jour. Nutr. 8: 731–749.

(6) Smith, Margaret Cammack, and Briggs, Ian A.
1933. The antirachitic value of alfalfa as affected by exposure to sunshine in the curing process. Jour. Agr. Res. 46: 235–240.

(7) Wallis, G. C.
1938. A comparison of the vitamin D potency of the stemmy and leafy portions of alfalfa hay. Jour. Agr. Res. 57: 393–396.

MEIOTIC BEHAVIOR OF PHLEUM PRATENSE, PHLEUM SUBULATUM, AND THEIR F_1 HYBRID [1]

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INTRODUCTION

The somatic chromosome number 42 has been reported for the American or "cultivated" timothy (*Phleum pratense* L.) by Gregor and Sansome (*4*),[2] by Avdulow (according to Müntzing (*11*)), by Müntzing (*11*), and by Nordenskiöld (*18*). Since 14- and 28- chromosome species of *Phleum* are known, Müntzing (*11*) concluded that the basic number of the genus is 7 and that *P. pratense* is, consequently, a hexaploid. Gregor and Sansome (*4*) and Gregor (*3*) obtained 4 hexaploid plants from seed produced on triploid F_1 plants of the hybrid *Phleum pratense* ($2n=14$) $\times$ *P. alpinum* ($2n=28$). One of these plants was cross-fertile with hexaploid *P. pratense*. On the basis of these results and the chromosome behavior in some plants that he considered to be derivatives of the hybrid *P. pratense* ($2n=42$) $\times$ *P. pratense* ($2n=14$), Müntzing (*11*) concluded that hexaploid *P. pratense* had the genomatic constitution *NNAABB*, where *N*, *A*, and *B* were different genomes of 7 chromosomes. This conclusion is further supported by Müntzing's (*11*) observation of 21 bivalents at metaphase I in *P. pratense* and by Nordenskiöld's (*18*) statement that multivalent associations were not found in this species. In a later paper, Müntzing and Prakken (*13*) did not exclude the possibility of quadrivalent formation but stated that such association was very rare. On the other hand, Nordenskiöld (*18*) reported that 14 bivalents occurred at metaphase I in hybrids of *P. pratense* ($2n=42$) $\times$ *P. pratense* ($2n=14$), indicating at least partial homology between the chromosomes of 2 of the genomes of hexaploid timothy. This conclusion was supported by the meiotic behavior in 63-chromosome plants of *P. pratense* that Müntzing and Prakken (*13*) obtained from twin seedlings. These plants had each genome represented 3 times and, as a result, might have been expected to show a considerable frequency of trivalents and a total of 21 trivalents and bivalents. Instead, the authors reported that trivalents were rare and that the sum of the trivalents and bivalents was seldom less than 28. On the basis of these results, they concluded that the genomatic constitution of *P. pratense* is $NNA_1A_1A_2A_2$, where A_1 and A_2 are sufficiently homologous to permit formation of bivalents between chromosomes of the 2 genomes.

The object of the investigation reported in this paper was to obtain further information relative to the genomatic constitution of hexaploid *Phleum pratense* and the interspecific relationships in the genus *Phleum*. The work was done at the United States Regional Pasture Research Laboratory,[3] State College, Pa.

[1] Received for publication January 27, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 658.

[3] Maintained in cooperation with the agricultural experiment stations of the 12 Northeastern States.

MATERIAL AND METHODS

Panicles of a male sterile plant of *Phleum pratense* were dusted with pollen from a plant of *P. subulatum* (Savi) Aschers. and Graebn., and three seeds were obtained. All the seeds germinated, and two plants grew to maturity. The third plant was lost, owing to an accident of greenhouse culture rather than to any inherent weakness. Plants from the clone of the *P. pratense* parent and from seed produced under bag on the *P. subulatum* parent were grown in the greenhouse at the same time as the hybrid plants for use in comparative studies.

Chromosome numbers of the parents and F_1 were determined from paraffin sections of root tips prepared in the same manner as described previously for *Lolium perenne* L. (*14*). For meiotic studies, fresh acetocarmine smear slides were prepared from material fixed in acetic alcohol.

EXPERIMENTAL RESULTS

MEIOSIS IN PHLEUM SUBULATUM

Determinations from root-tip preparations and from microsporocytes showed that *Phleum subulatum* was diploid ($2n=14$). Thirty-six sporocytes at diakinesis and 149 sporocytes at metaphase I were examined, and, of these, 183 (98.9) percent had 7 bivalents. In each of 2 sporocytes (1.1 percent of those examined) 2 univalents and 6 bivalents were found. The total chiasma frequency in 20 sporocytes at metaphase I ranged from 10 to 15, with an average of 12.95 per sporocyte, or 1.85 per bivalent. The average frequency of terminal chiasmata was 12.5, giving a terminalization coefficient of 0.96. As indicated from these data, the majority of bivalents had 2 chiasmata, 1 in each arm, resulting in ring-shaped bivalents. However, on an average, 1.35 bivalents per sporocyte were of the open type, i. e., with a single chiasma. A sporocyte at metaphase I with 7 ring-shaped bivalents is shown in figure 1, *B*, while the sporocyte in figure 1, *C*, has 2 open bivalents. Figure 1, *A*, shows a sporocyte at diakinesis with 7 ring-shaped bivalents. In this sporocyte 1 bivalent lies on the nucleolus, while the remaining 6 are free from it. Since this was a regular occurrence at diakinesis, it is probable that only 1 member of the haploid complement bears a nucleolus organizer, although this conclusion could not be verified by studies of midprophase.

Of 122 sporocytes examined at anaphase I, none showed lagging univalents, the distribution in all cases being 7 chromosomes to each pole. Likewise, no evidence of chromosome loss was found at interphase I. At anaphase II some chromosomes lagged; these were left in the cytoplasm in one or more cells of 2.47 percent of the 202 quartets observed. The origin of the lagging univalents at anaphase II was not determined. In other species such laggards frequently are daughter univalents from chromosomes that had lagged and divided equationally at anaphase I. Although no laggards were observed at anaphase I in this material, it is possible that examination of a larger number of sporocytes would have revealed some lagging univalents.

MEIOSIS IN PHLEUM PRATENSE

Attempts to study midprophase in *Phleum pratense* were only partially successful. Pairing appeared to be predominantly as bivalents, although occasional exchanges of partners were observed. It

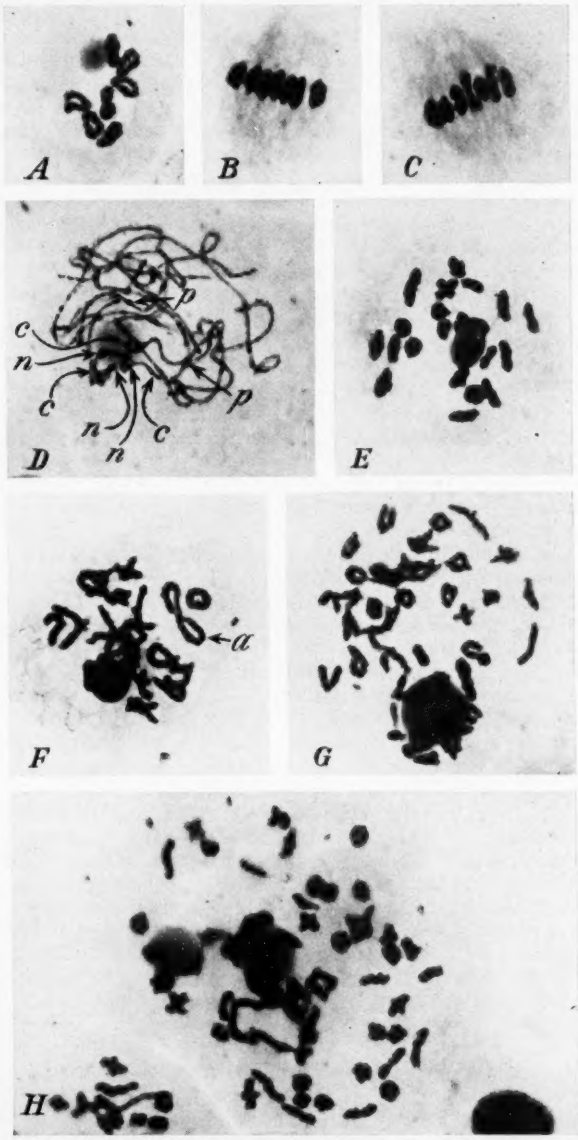

FIGURE 1.—*A*, Diakinesis in *Phleum subulatum; B*, metaphase I in *P. subulatum* with 7 ring-shaped bivalents; *C*, metaphase I in *P. subulatum* with 2 open bivalents; *D*, midprophase in *P. pratense*, the arrows at *n* indicating nucleolus organizers; *c*, centromere; and *p*, pycnotic knobs; *E*, diakinesis in *P. pratense* with 21 bivalents; *F*, diakinesis in *P. pratense* showing quadrivalent at *a*; *G*, diakinesis in sporocyte of *P. pratense* with 84 chromosomes; *H*, diakinesis in sporocyte of *P. pratense* with 126 chromosomes. × 850.

was not possible to analyze a complete nucleus in any case. Figure 1, *D*, shows the most satisfactory preparation obtained. Three chromosomes were attached to the nucleolus by dark-staining bodies (indicated by arrows at *n* in fig. 1, *D*) similar in appearance to the nucleolus organizer reported in maize by McClintock (*10*). The nucleolus organizer was located in the short arm of each chromosome approximately midway between the centromere (located at *c* in fig. 1, *D*) and the end of the chromosome. These three nucleolar chromosomes appeared to be approximately alike in total length and in relative length of the two arms. Three other chromosomes each had a pycnotic knob (two shown at *p* in fig. 1, *D*) similar to the knobs on the maize chromosomes (*9*). Likewise, these three chromosomes were alike in total length and arm length ratio. Apparently at least two of the seven chromosomes in each genome are similarly represented in each of the three genomes.

The type of chromosomal association was determined in 72 sporocytes at diakinesis. Of these, 33.3 percent had 21 bivalents (fig. 1, *E*); 2.8 percent had 20 bivalents plus 2 univalents; 34.7 percent had 19 bivalents plus 1 quadrivalent (fig. 1, *F*, the quadrivalent at *a*); 23.6 percent had 17 bivalents plus 2 quadrivalents; and 5.6 percent had 15 bivalents plus 3 quadrivalents. In addition to these 72 sporocytes, many more were examined for which a complete analysis was not possible. Among these a few sporocytes with 4 quadrivalents were seen, but none with more than 4 was found.

It was not possible with the material available to make an accurate determination of chiasma frequency, but it appeared to be relatively low. About one-half of the bivalents were of the open type with a chiasma in only one arm. In a majority of the bivalents the chiasmata were terminal or subterminal, but in others they were clearly interstitial (fig. 1, *E* and *F*). Whether these latter cases indicate a partial tendency for localization of the chiasmata could not be determined.

Two sporocytes at diakinesis were found that had 84 and 126 chromosomes respectively (fig. 1, *G* and *H*). Apparently these sporocytes had resulted from chromosomal reduplication in premeiotic divisions. In the former the chromosomal association was $1_{VIII}+4_{IV}+30_{II}$, while in the latter it was $1_{VIII}+3_{IV}+53_{II}$. Although each of the 3 genomes must have been present 4 and 6 times respectively in the 2 sporocytes, pairing was still predominantly as bivalents, indicating that polyvalent association does not necessarily follow in this species as a result of the presence of more than 2 homologous chromosomes.

In the sporocytes that were examined at diakinesis, at least three bivalents or one bivalent and one quadrivalent were always touching the nucleolus. This would be expected from the observation at midprophase of three chromosome pairs with nucleolus organizers.

MEIOSIS IN PHLEUM PRATENSE × P. SUBULATUM

From root-tip preparations the chromosome number of the 2 surviving plants was found to be 28, confirming the supposition that they were hybrids that had resulted from the union of reduced gametes from the parental species.

The frequency of bivalents and univalents was determined in 21 sporocytes at metaphase I. These varied from 7 bivalents plus 14 univalents to 12 bivalents plus 4 univalents, averaging 9.43 bivalents and 9.05 univalents per sporocyte. Of the bivalents observed, 41.9 percent were of the open type as compared with 19.3 percent of the

open type in *Phleum subulatum*. The average chiasma frequency was 1.66 per bivalent in the hybrid as compared with 1.85 in *P. subulatum*. In addition to the sporocytes that were analyzed in detail, numerous others were examined, and none was found with less than 4 univalents. Also, no associations other than univalents or bivalents were observed. The univalents were oriented along with the bivalents in a regular manner on the equatorial plane (fig. 2, *A*). Only rarely was a univalent found lying in the cytoplasm some distance from the metaphase plate (fig. 2, *B*).

Lagging univalents that were undergoing equational divisions were found in all anaphase I sporotcyes that were observed. Two anaphase I sporocytes are shown lying adjacent to metaphase I sporocytes in figure 2, *B*; while figure 2, *C*, shows adjacent anaphase I and anaphase II sporocytes. The average frequency of lagging and dividing univalents in 21 anaphase I sporocytes was 8.9, a value slightly but probably not significantly lower than the average number of unpaired chromosomes at metaphase I. Apparently most of the metaphase I univalents lag and divide at anaphase I. A dicentric bridge and acentric fragment (fig. 2, *D*) were found in 2 of the 21 anaphase I sporocytes, while in 1 cell 2 dicentric bridges and 2 acentric fragments occurred. Chromatin bridges and acentric fragments were also seen in sporocytes at telophase I (fig. 2, *E*), although the frequency at this stage was not determined.

Studies of interphase I indicated that a majority of the daughter half chromosomes from lagging and dividing univalents at anaphase I arrived at the poles in time to be included in the daughter nuclei. If none of the half chromosomes from the anaphase I laggards were included in the daughter nuclei, the frequency of chromosomes in the cytoplasm at interphase I should be twice the frequency of lagging univalents at anaphase I. Instead, no chromosomes were found in the cytoplasm in 26.8 percent of the interphase I sporocytes examined. The greatest number of chromosomes in the cytoplasm in any sporocyte was five, two in one cell and three in the other, or one in one cell and four in the other. Only 7.3 percent of the sporocytes were of these two types. A chromatin bridge and acentric fragment were found in 14.6 percent of the interphase I sporocytes. This value approximates closely the 14.3 percent of anaphase I sporocytes with dicentric bridges and acentric fragments.

At metaphase II a majority of the daughter univalents had congressed in a normal manner, being oriented on the metaphase plate along with the chromosomes that had resulted from disjunction of the metaphase I bivalents. However, the congression was not complete in all sporocytes. In 89.1 percent of the 46 sporocytes examined, 1 or more daughter univalents were lying at some distance from the metaphase II plate (fig. 2, *F*). In most cases these daughter univalents were near one of the poles and apparently were in a position to be included in one of the quartet nuclei.

Lagging univalents occurred in all anaphase II sporocytes that were observed (fig. 2, *C*). Owing to a tendency for these univalents to clump badly, exact determinations of their number were impossible except in a few sporocytes. In these, the number of laggards varied from 7 to 12 in each cell of the sporocyte. It seems probable that these laggards were the daughter univalents from anaphase I laggards.

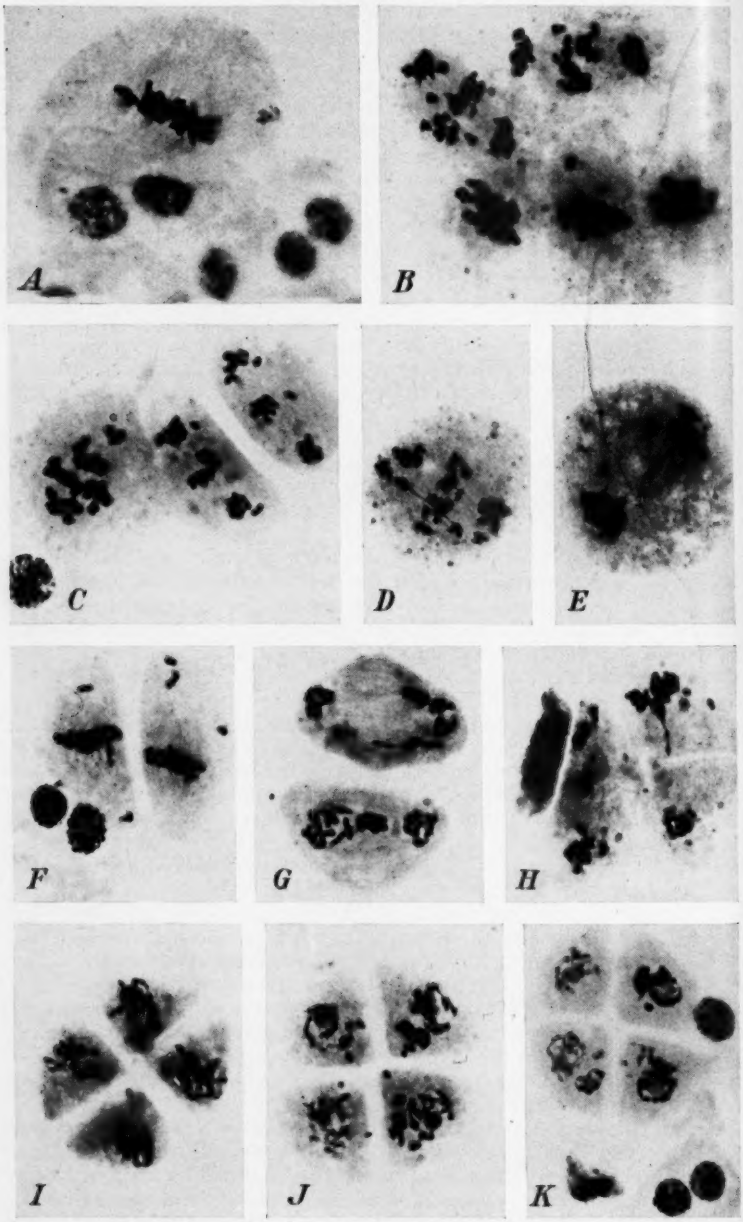

FIGURE 2.—Meiosis in *Phleum pratense* × *P. subulatum: A*, Metaphase I; *B*, adjacent metaphase I and anaphase I sporocytes; *C*, adjacent anaphase I and anaphase II sporocytes; *D*, anaphase I with dicentric bridge; *E*, telophase I with dicentric bridge and acentric fragment; *F*, metaphase II; *G*, telophase II; *H*, *I*, *J*, *K*, quartets. × 850.

The data on their frequency were too limited to permit a determination of what proportion of the daughter univalents lagged at anaphase II.

Quartet formation was so irregular that statistical analysis was not possible. Chromatin clumps or micronuclei were found in all quartets (fig. 2, *G* to *K*). Some of these chromatin clumps appeared to be single daughter univalents, while others apparently consisted of two or more univalents that had clumped together. Sporocytes in which the univalents formed a micronucleus midway between the quartet nuclei (fig. 2, *G*) were common. Frequently this chromatin clump had been cut in two by the cell wall formation (fig. 2, *I*), but in other sporocytes wall formation was inhibited, resulting in two or three cells instead of the normal four. In rare instances one cell of the quartet had a normal-appearing nucleus not accompanied by micronuclei or chromatin clumps (fig. 2, *K*).

MORPHOLOGY AND FERTILITY

The parental species used in this study have been described by Hitchcock (*5*). Except in respect to the following three characteristics,

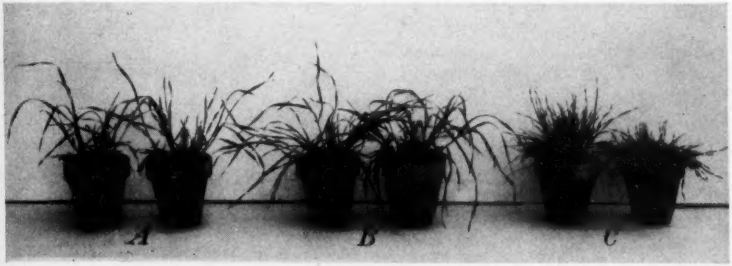

FIGURE 3.—*A, Phleum pratense; B, P. pratense* × *P. subulatum; C. P. subulatum.*

the F_1 plants were indistinguishable morphologically from *Phleum pratense*, as might be expected from the fact that this parent contributed 21 of the 28 chromosomes. (1) Prior to the initiation of flowering culms the hybrid plants had more basal leaves than the *P. pratense* parent (fig. 3), but since variability in this character occurs between pots of the same clone under greenhouse conditions, and since the comparison was between hybrid plants from seed and parental plants from clones, this difference may not be significant. (2) The hybrid plant maintained greater leaf growth during and following seed production than either parent. *P. pratense*, under both field and greenhouse culture, has sparse leaf growth at this stage, while *P. subulatum* is an annual and dies as soon as the seed is mature. (3) In the red coloring of the glumes and peduncles the hybrids were intermediate between *P. pratense*, which lacked such coloring, and *P. subulatum*, on which the glumes and peduncles were dark red, almost purple.

The hybrid plants were completely male sterile, as might be expected from their meiotic behavior. Young microspores rarely appeared normal, and the anthers were never observed to dehisce. Three panicles of the hybrid were enclosed under bags for selfing, but no seeds were set. Eight other panicles were pollinated with pollen from normal *P. pratense*, and 27 seeds, an average of 3.38 seeds per panicle, were obtained.

DISCUSSION

The bivalent frequency in the hybrid plants could be accounted for in 3 ways: (1) One genome of *Phleum pratense* is homologous with the genome of *P. subulatum*, and the other 2 genomes of *P. pratense* are partially homologous; (2) only partial homology exists between 1 genome of *P. pratense* and the genome of *P. subulatum*, while the other 2 genomes of *P. pratense* are completely homologous; (3) only partial homology exists between 1 *P. pratense* genome and the genome of *P. subulatum* as well as between the 2 remaining genomes of *P. pratense*. It is impossible from the data available to determine which of these explanations is the correct one. Some evidence for the first is that the maximum number of quadrivalents observed in *P. pratense* was 4, while the maximum number of bivalents in the hybrid was 12, or 5 more than would be accounted for by pairing between *P. pratense* and *P. subulatum* chromosomes.

On the basis of the results reported by Nordenskiöld (*18*) and Müntzing and Prakken (*13*), the second explanation seems more logical. However, if the hypothesis of complete homology between chromosomes of two genomes of *Phleum pratense* is accepted, it is necessary to account for the low frequency of quadrivalent formation in normal plants of this species. This question has been discussed thoroughly by Müntzing and Prakken (*13*). Little further discussion is warranted at present except to point out that in the plant of *P. pratense* used in this study the quadrivalent frequency was higher and the chiasma frequency lower than in the material used by Nordenskiöld (*18*) or by Müntzing and Prakken (*13*). Obviously, a more extensive study of plants of *P. pratense* is desirable.

Müntzing and Prakken (*13*) raised the question whether the chromosomes of the two homologous genomes of *Phleum pratense* normally associate as bivalents in particular pairs or whether bivalent formation was at random among each set of four homologues. Critical cytological evidence cannot be obtained on this question. On the other hand, the genetical consequences of the two types of pairing will be different. There is, however, a paucity of genetical data for *P. pratense*. Barker and Hayes (*1*) obtained 3:1 ratios for reaction to rust among inbred progenies, and Proytchoff (according to Horsfall (*6*)) obtained similar results. Likewise, Myers and Chilton (unpublished data), in studies of hybrid populations involving rust-resistant parents, obtained ratios of 3:1 in crosses of resistant × resistant, and ratios of 1:1 in crosses of resistant × susceptible plants. Unfortunately, this evidence is negative, and limited negative evidence from progenies of plants from open-pollinated populations is useless in solving this particular problem, since an allotetraploid of the constitution $A_1a_1a_2a_2$ will produce progenies in the same phenotypic ratios as will an autotetraploid of the constitution *Aaaa*.

The behavior of the univalents at metaphase I in the hybrid plants reported in this paper differs from the usual behavior of unpaired chromosomes. According to Darlington (*2*), Jenkin and Thomas (*7*), Levan (*8*), and others, univalents usually lie outside of the spindle at metaphase I and either do not congress or do so later than the bivalents. In the *Phleum pratense* × *P. subulatum* hybrid the univalents were lying in the equatorial plane along with the bivalents. In this respect

the behavior resembles that reported by Levan (*8*) for the univalents in asynaptic *Allium amplectens*.

In his studies of a telocentric chromosome in maize, Rhoades (*20*) suggested that the univalents that congressed divided equationally at anaphase I. This conclusion is supported by the results obtained by the writer. In the hybrid reported in this paper, the univalents had congressed at metaphase I, and the average frequency of dividing univalents at anaphase I was almost equal to the average number of unpaired chromosomes at metaphase I. On the other hand, in *Lolium perenne*, Myers (*15*) found a lower frequency of dividing univalents at anaphase I than of unpaired chromosomes at metaphase I. In these plants the congression of univalents was not complete.

The occurrence of two dicentric bridges and acentric fragments at anaphase I in the hybrid plants indicates that at least two of the bivalents were heterozygous for an inversion. These inversions could have been present either in the bivalents resulting from pairing of *Phleum pratense* and *P. subulatum* chromosomes or in those resulting from autosyndesis of *P. pratense* chromosomes. In either case the presence of inversions does not necessarily represent a phase in the differentiation between the two species or between genomes of *P. pratense*. Müntzing (*12*), Myers and Hill (*16, 17*), Myers (*15*), and Östergren (*19*) have reported the common occurrence of plants of normally cross-pollinated species that are heterozygous for inversions. It was not determined whether or not the *P. pratense* plant used in this investigation was heterozygous for one or more inversions.

SUMMARY

The 14 chromosomes of *Phleum subulatum* normally were associated as 7 bivalents at diakinesis and metaphase I. In 1.1 percent of the sporocytes 6 bivalents plus 2 univalents were present. The average chiasma frequency at metaphase I was 1.85 per bivalent. Anaphase I was regular, but at anaphase II some lagging univalents occurred, resulting in chromosomes being left in the cytoplasm in 2.47 percent of the quartets.

In *Phleum pratense*, at diakinesis, 33.3 percent of the sporocytes had 21 bivalents and 2.8 percent had 20 bivalents plus 2 univalents. The remaining 63.9 percent had 1 to 3 quadrivalents plus 19 to 15 bivalents. Four quadrivalents were observed rarely. The chiasma frequency was low, and about one-half of the bivalents had a chiasma in only 1 arm.

The hybrid plants (*Phleum pratense* × *P. subulatum*) had 28 chromosomes. At metaphase I the chromosomal association ranged from 7 bivalents plus 14 univalents to 12 bivalents plus 4 univalents, averaging 9.43 bivalents and 9.05 univalents per sporocyte. The univalents were oriented in a regular manner on the equatorial plane. Lagging univalents undergoing equational division were found in all anaphase I sporocytes, the average being 8.9. At metaphase II a majority of the daughter univalents congressed normally but did not divide at anaphase II. Laggards occurred at anaphase II in all sporocytes, resulting in irregular quartet formation.

Morphologically the hybrid closely resembled *Phleum pratense*. The hybrid was completely male sterile, since the anthers did not dehisce, but was partially female fertile with *P. pratense* pollen.

LITERATURE CITED

(1) Barker, H. D., and Hayes, H. K.
1924. rust resistance in timothy. Phytopathology 14: [363]–371, illus.
(2) Darlington, C. D.
1937. recent advances in cytology. Ed. 2, 671 pp., illus. Philadelphia.
(3) Gregor, J. W.
1931. experimental delimitation of species. New Phytol. 30: 204-217, illus.
(4) ——— and Sansome, F. W.
1930. experiments on the genetics of wild populations. II. phleum pratense l. and the hybrid p. pratense l. × p. alpinum l. Jour. Genet. 22: [373]–387, illus.
(5) Hitchcock, A. S.
1935. manual of the grasses of the united states. U. S. Dept. Agr. Misc. Pub. 200, 1,040 pp., illus.
(6) Horsfall, James G.
1930. a study of meadow crop diseases in new york. N. Y. (Cornell) Agr. Expt. Sta. Mem. 130, 139 pp., illus.
(7) Jenkin, T. J., and Thomas, P. T.
1939. interspecific and intergeneric hybrids in herbage grasses. III. lolium loliaceum and lolium rigidum. Jour. Genet. 37: [255]–286, illus.
(8) Levan, Albert.
1940. the cytology of allium amplectens and the occurrence in nature of its asynapsis. Hereditas 26: [353]–394, illus.
(9) McClintock, Barbara.
1933. the association of non-homologous parts of chromosomes in the mid-prophase of meiosis in zea mays. Ztschr. f. Zellforsch. u. Mikros. Anat. 19: [191]–237, illus.
(10) ———
1934. the relation of a particular chromosomal element to the development of the nucleoli in zea mays. Ztschr. f. Zellforsch. u. Mikros. Anat. 21: [294]–328, illus.
(11) Müntzing, Arne.
1935. cyto-genetic studies on hybrids between two phleum species. Hereditas 20: [103]–136, illus.
(12) ———
1939. chromosomenaberrationen bei pflanzen und ihre genetische wirkung. Ztschr. f. Induktive Abstamm. u. Vererbungslehre 76: [323]–351, illus.
(13) ——— and Prakken, R.
1940. the mode of chromosome pairing in phleum twins with 63 chromosomes and its cytogenetic consequences. Hereditas 26: 463–501, illus.
(14) Myers, W. M.
1939. colchicine induced tetraploidy in perennial ryegrass, lolium perenne l. Jour. Hered. 30: 499–504, illus.
(15) ———
1941. variations in chromosomal behavior during meiosis among plants of lolium perenne l. Cytologia 11: 388–406, illus.
(16) ——— and Hill, Helen D.
1940. the association and behavior of chromosomes in autotetraploid grasses. (Abstract) Genetics 25: 129.
(17) ——— and Hill, Helen D.
1940. studies of chromosomal association and behavior and the occurrence of aneuploidy in the autotetraploid grass species, orchard grass, tall oat grass, and crfsted wheatgrass. Bot. Gaz. 102: 236–255, illus.

(18) NORDENSKIÖLD, HEDDA.
1937. INTRA- AND INTERSPECIFIC HYBRIDS OF PHLEUM PRATENSE AND P. ALPINUM. Hereditas 23: [304]–316, illus.
(19) ÖSTERGREN, G.
1940. CYTOLOGY OF AGROPYRON JUNCEUM, A. REPENS AND THEIR SPONTANEOUS HYBRIDS. Hereditas 26: [305]–316, illus.
(20) RHOADES, M. M.
1940. STUDIES OF A TELOCENTRIC CHROMOSOME IN MAIZE WITH REFERENCE TO THE STABILITY OF ITS CENTROMERE. Genetics 25: [483]–520, illus.

GENETIC STUDIES OF VARIEGATION IN SNAP BEANS[1]

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INTRODUCTION

Since the introduction of Wisconsin Refugee and Idaho Refugee beans (*Phaseolus vulgaris L.*) in 1934 there have been many comments concerning variegation in these varieties (*4*)[2 3 4] and a partial genetic explanation of this or a related condition (*7*). The occurrence of variegations in certain breeding stocks at the United States Regional Vegetable Breeding Laboratory at Charleston, S. C., has made it necessary to give further attention to the problem of variegation.

MATERIAL AND METHODS

In the spring of 1936 a variegation occurred in a stock of U. S. No. 5 Refugee. This plant was undoubtedly of hybrid origin, because many of its characteristics were different from U. S. No. 5 Refugee, and its progeny segregated for seed color, pod color (wax and green), size of pods, and plant size. This material, which was of value for breeding purposes, was later used in crosses.

As variegation normally does not occur in U. S. No. 5 Refugee, it is possible that the variegated plant came from a mechanical mixture of some hybrids derived from crosses with strains of Corbett Refugee. Wisconsin Refugee and Idaho Refugee, which normally show variegation, were derived from crosses of Stringless Green Refugee with Corbett Refugee, and in many cases the hybrids carried by the United States Department of Agriculture at Greeley, Colo., showed variegation if Corbett Refugee was one of the parents.

The pure line of the variegated strain was crossed reciprocally with Black Valentine, and observations were made in the F_1, F_2, F_3, and F_4 generations. As previous experience with variegation in Corbett Refugee had shown that it was difficult to evaluate, the experiment was set up in such a way as to control variability as much as possible. The F_1 and F_2 plants were covered with cheesecloth at blooming time to shut out insects, and notes were taken at least three times during the life of a plant to be sure that the information obtained was accurate. F_1 plants were spaced 3 feet apart in 3-foot rows, F_2 seeds 1 foot apart, and F_3 and F_4 seeds 6 inches apart in 3-foot rows. The smallest F_3 family consisted of 39 plants and the average number per F_3 and F_4 family was over 60 plants.

[1] Received for publication February 13, 1941. This work was performed under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] Italic numbers in parentheses refer to Literature Cited, p. 669.

[3] HARRISON, A. L., and BURKHOLDER, W. H. CANNING BEAN DISEASES IN NEW YORK IN 1936. U. S. Bur. Plant Indus., Plant Dis. Rptr. 20: 290-291. 1936. [Mimeographed.]

[4] HORSFALL, JAMES G., BURKHOLDER, W. H., and REINKING, O. A. DISEASES OF GREEN REFUGEE BEANS IN NEW YORK IN 1937. U. S. Bur. Plant Indus., Plant Dis. Rptr. 21: 318-319. 1937. [Mimeographed.]

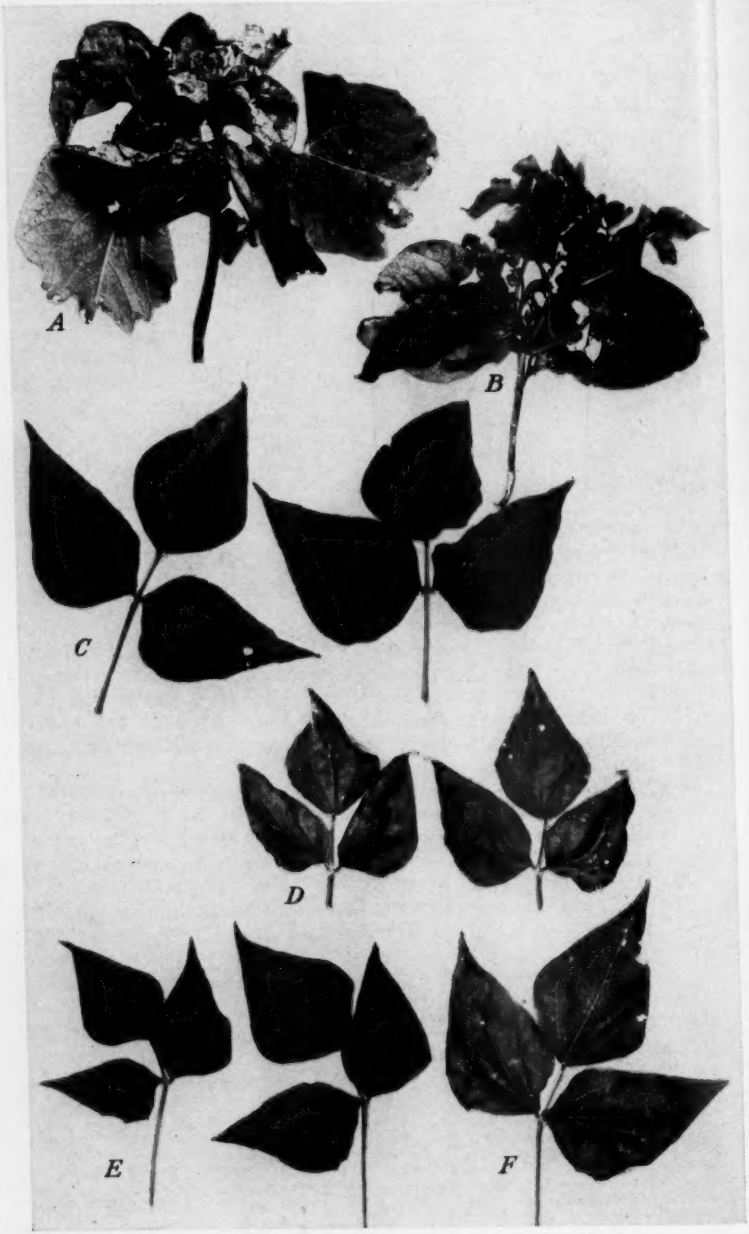

FIGURE 1.—For explanatory legend see opposite page.

All plants were field-grown except part of those grown to study the influence of environment on variegation (see table 3). The seeds for these tests were germinated on blotters and transferred to greenhouse soil as soon as roots had reached a length of about an inch. The plants were spaced 6 inches apart in rows 12 inches wide, and up to blooming time three sets of notes were taken on degree of variegation. In compiling the data the greatest degree of variegation noted for a plant was used.

The variegation discussed herein ranged from very severe (fig. 1, *A*) to very mild (fig. 1, *E*). Variegation rarely occurred in the primary leaves, but any trifoliate leaves produced up to blooming time might show it. After blooming the variegation tended to become less distinct, and only rarely did any leaves produced after blooming show it. The very severe type was not common, but the severe type was very common. The severe type differed from the very severe in that normal or nearly normal branches were put out by plants with the severe type and they produced normal crops, whereas the plants labeled very severe usually died before any pods were produced. In plants with the very severe and severe types the variegation ranged from a distinct chimera with deformity in some leaves to a mild flecking in other leaves (fig. 1, *A* and *B*). Plants of the medium, mild, and very mild types had one or more variegated leaflets, the variegation decreasing in intensity as shown in figure 1, *C* to *E*. Sometimes considerable deformity occurred, but usually only in the first two classes.

In all plantings the two parents were carried as checks. Because of the size of families it was not considered desirable to replicate, but the parents were planted at random and no effect of field location on expression of variegation was noticed.

All ratios were subjected to chi-square analysis according to the methods of Fisher (*1*), using Yates' correction for continuity, and of Mather (*3*), except that in testing for the significance of chi square beyond 30 degrees of freedom the approximation of Wilson and Hilferty (*6*) was used. Continuity corrections were applied to all ratios having 1 degree of freedom for chi-square computations, but for accumulated chi-square values (see tables 1 and 2) uncorrected values were added.

RESULTS

The F_1 plants from the cross of the variegated strain with Black Valentine and from the reciprocal showed no variegation. The F_1 plants were so prolific that only two plants were necessary to obtain sufficient F_2 seed.

From the F_2 plants 2 sets of results were recorded, 1 from the cross in which the variegated strain was used as the female parent (designated line A) and the other from the reciprocal (designated line B). Both lines segregated in a ratio of approximately 27 normal plants to

EXPLANATORY LEGEND FOR FIGURE 1.

FIGURE 1.—Types of variegation found under growing conditions favorable for the variegated parent: *A*, Very severe type; *B*, severe type; *C*, medium type, with very little deformity and most leaves with none; *D*, mild type; *E*, very mild type; *F*, normal (nonvariegated) leaf.

37 variegated. Table 1 shows the results from the two lines separately and in combination. The combined ratio is 293 normal to 369 variegated plants, with a nonsignificant chi-square value.

From the F_2 plants 220 of line A and 215 of line B were harvested for seed from the field as they occurred, without selection. From these were produced 124 and 122 variegated families of lines A and B, respectively. These 246 families were composed of all variegated plants except for 2 families from line A and 1 from line B, which had 1 or 2 plants each in which variegation could not be detected. These 5 plants were progeny-tested and found to be pure for the variegated condition.

From the 96 normal F_2 plants of line A were obtained 5 F_3 families in which all plants were normal, 25 with a ratio of 3 normal to 1 variegated, 40 with a ratio of 9 normal to 7 variegated, and 26 with a ratio of 27 normal to 37 variegated. From the 93 normal F_2 plants of line B were obtained 4 F_3 families in which all plants were normal, 22 with a 3:1 ratio, 38 with a 9:7 ratio, and 29 with a 27:37 ratio. Chi-square tests applied to these data for lines A and B, singly and combined, show no significant deviation from expectation for a 3-factor ratio of this kind (table 1).

TABLE 1.—*Results obtained with F_2 and F_3 progenies from the cross variegated × Black Valentine (line A) and the reciprocal (line B)*

[Degrees of freedom not indicated unless more than 1]

PARENTS

Line	Normal plants	Variegated plants
	Number	*Number*
Variegated	[1] 2	1,121
Black Valentine	1,063	[1] 1

F_2 GENERATION

Line	Normal plants	Variegated plants	χ^2 based on theoretical 27:37 ratio
	Number	*Number*	
A	159	180	2.900
B	134	189	.040
Total	293	369	1.082

F_3 FAMILIES [3]

Line	All normal	Segregating as indicated			All variegated	χ^2 based on theoretical 1:6:12:8:37 ratio	Degrees of freedom
		3:1	9:7	27:37			
	Number	*Number*	*Number*	*Number*	*Number*		*Number*
A	5	25	40	26	124	1.838	4
B	4	22	38	29	122	.634	4
Total	9	47	78	55	246	1.945	4

See footnotes at end of table.

TABLE 1.—*Results obtained with F_2 and F_3 progenies from the cross variegated × Black Valentine (line A) and the reciprocal (line B)*—Continued

F_3 SEGREGATING PROGENIES [4] [5]

Segregation ratio and line	Families	Accumulated χ^2	Degrees of freedom	Normal plants	Variegated plants	Population χ^2
3 : 1 ratio:	*Number*		*Number*	*Number*	*Number*	
A	25	27.321	25	1,184	359	2.382
B	22	34.166*	22	960	260	8.657**
Total	47	61.487	47	2,144	619	9.699**
9 : 7 ratio:						
A	40	38.850	40	1,510	1,053	6.815**
B	38	43.562	38	1,505	1,023	10.940**
Total	78	82.412	78	3,015	2,076	18.154**
27 : 37 ratio:						
A	26	29.798	26	760	880	11.433**
B	29	33.608	29	837	1,016	6.636**
Total	55	63.406	55	1,597	1,896	17.727**

[1] Progeny test showed these to be variegated.
[2] Progeny test showed this to be normal.
[3] Separation into F_3 groups shown based on F_3 data, supplemented with F_4 data for all doubtful cases.
[4] All F_3 progenies with chi-square values in excess of 1.000 were studied in F_4.
[5] *=Significant at 5-percent level. **=Significant at 1-percent level. All heterogeneity chi squares (accumulated less population chi square) occur within the range of 5-percent to 95-percent probability. Continuity correction was applied to chi-square values with 1 degree of freedom, but added chi-square values of 1 degree of freedom each for accumulated chi square are uncorrected.

The F_3 segregating families when combined for chi-square tests for populations showed a deficiency of variegated plants. Chi square was highly significant in all cases except for the 3:1 families combined for line A (table 1).

The accumulated chi-square values for the various families indicate that families showing like segregations had been combined (table 1). Heterogeneity chi squares are not shown in the table, but in all cases when they are computed by subtracting chi square for the F_3 population from the corresponding accumulated chi square the values were within the range of 5-percent to 95-percent probability.

As ratios requiring large numbers for satisfactory tests are difficult to work with on account of the inability of many plants to set a large number of seeds, it was decided to give further attention to simpler tests requiring smaller numbers. Theoretically, in inheritance of the kind described in this paper there should be three types of variegated plants arising in connection with the 3:1 ratios. If two of the same type are crossed the F_1 plants should all be variegated and there should be no segregation in the F_2 generation, whereas if two of different types are crossed the F_1 plants should be normal and the F_2 segregation should be 9 normal to 7 variegated. Three types of variegated plants as postulated were recovered and tested in F_1 and F_2. All F_1 plants were normal. The F_2 results are given in table 2. All the ratios showed nonsignificant deficiency of variegated plants, but when the population chi square was computed it exceeded the 5-percent level of probability.

The three variegated types (*a*, *b*, and *c*) recovered from the 3:1 ratios were very difficult to distinguish from each other, but on the basis of the most fully variegated plants from each line they were tentatively described. Type *a* had a yellow tinge, type *b* was almost white in the variegated areas, and type *c* was intermediate or light

yellow. Crosses between these types showed in the 9:7 F_2 ratios about the same range in amount of variegation as did the 3:1 ratios, but on an average the variegation covered a greater amount of leaf surface. The three 9:7 types of ratios obtained by crossing *a*, *b*, and *c* in all possible combinations were not distinguishable from each other. The 27:37 ratio gave variegated plants with somewhat greater variegation than did the 9:7 ratio. By taking advantage of this fact it was possible to distinguish in a preliminary way the 3:1, 9:7, and 27:37 families in F_3. By selecting for the most fully variegated plants in families segregating 27:37 it was possible to recover the triple recessive. This averaged heavily variegated with a slight yellowish tinge, and in crosses with types *a*, *b*, and *c* it always produced a variegated F_1 and no segregation in F_2. On the basis of variegation it could not be distinguished from the original variegated parent.

TABLE 2.—*Variegated and normal F_2 plants from crossing three types (a, b, and c) of variegated F_3 segregates from a cross of Black Valentine × variegated*

Cross number and variegation types crossed	Normal plants	Variegated plants	Chi square based on theoretical 9:7 ratio
	Number	*Number*	
1 (*a*×*b*)	104	76	0.171
2 (*b*×*a*)	94	62	1.018
3 (*a*×*c*)	106	65	2.288
4 (*c*×*a*)	84	60	.254
5 (*b*×*c*)	84	54	1197
6 (*c*×*b*)	102	71	.516
Total accumulated χ^2			5.444
Total population χ^2	574	388	[1] 4.427*
Heterogeneity χ^2 for 5 degrees of freedom			[2] 1.017

[1] *=Significant at 5-percent level of probability.
[2] 95-percent level of probability=1.145.

As the expression of variegation fluctuated so much, it was decided to study this under various conditions with selected strains. Sufficient seed of each strain was planted to insure at least 50 variegated plants in plantings made in the field in both early and late spring and in the greenhouse. Deformity of leaves was disregarded, and a scale of values from 1, with only a trace of variegation, up to 20, with about 25 percent of the total leaf surface variegated, was used. The parents, the recovered triple recessive, Corbett Refugee, and variegated plants from 3 lines of 3:1 and from 3 lines of 9:7 were observed. The best expression of variegation occurred under greenhouse conditions with temperatures between 70° and 80° F.; the next best occurred in the field under early-spring conditions (table 3). Late-spring planting in the field resulted in a reduction of the range as well as of the mean. Corbett Refugee was observed to be the most variable in expression of variegation. In field plantings of Corbett Refugee only about 25 percent showed variegation, and this was relatively slight. Under greenhouse conditions, however, all Corbett Refugee plants were variegated, and the mean was very close to the mean of the 3 lines carrying double recessives.

TABLE 3.—*Variegation in various types of variegated plants grown in three different environments*

[Variegation scale of 1 for trace to 20 for variegation on about 25 percent of leaf surface; 50 variegated plants of each strain]

Strain	Degree of variegation in—					
	Field planting in—				Greenhouse planting	
	Early spring		Late spring			
	Range	Mean	Range	Mean	Range	Mean
Black Valentine	0	0	0	0	0	0
Variegated (original parent)	1–19	12.0	1–12	7.0	2–20	14.3
Line of type *a* from 3 : 1 ratio	1–18	7.1	1–11	5.0	2–20	10.2
Line of type *b* from 3 : 1 ratio	2–18	7.4	1–11	5.3	2–20	10.1
Line of type *c* from 3 : 1 ratio	1–19	7.0	1–10	5.1	2–20	10.0
Triple recessive	1–19	12.4	1–12	7.5	2–20	14.0
Variegated from 9 : 7 ratio (*a*×*b*)	1–18	9.5	1–10	6.4	2–20	11.8
Variegated from 9 : 7 ratio (*a*×*c*)	1–18	9.0	1–11	6.0	2–20	11.6
Variegated from 9 : 7 ratio (*b*×*c*)	2–19	9.3	1–11	6.2	2–20	11.2
Corbett Refugee [1]	1–7	3.0	1–5	2.3	2–15	11.3
Significant difference		2.0		2.3		1.1

[1] Data for Corbett Refugee are based only on plants showing variegation (about 25 percent of plants in field; 100 percent of plants in greenhouse).

As over 26,000 plants were observed in the F_3 generation alone, it is reasonable to inquire to what extent misclassifications may have occurred. Table 1 shows that of 1,123 plants of the variegated parent line, 2 failed to show variegation, but all the progeny produced from these 2 plants were variegated. Of a total of 1,064 Black Valentine plants, 1 plant showed a slight loss of chlorophyll suspiciously like a mild variegation, but all the offspring were normal. If these errors tend to balance each other, then there is an error of classification of about 0.1 percent, or about 26 plants out of the 26,000 observed. Three F_3 of 246 variegated families gave 5 nonvariegated plants, all of which produced variegated progeny. Since the 246 families involved had approximately 15,000 plants, 15 plants would have been expected to be misclassified on the basis of 0.1 percent.

No natural crosses were observed except in the F_4 group of material, indicating that covering F_1 and F_2 plants with cheesecloth at blooming time had been effective in shutting out pollen-carrying insects.

DISCUSSION

The variegation reported here fluctuates a great deal in its expression, and no reason for this has been found. Despite this fluctuation, it has been possible to arrive at a factorial explanation. The normal condition is due to the complementary action of three dominant genes, and the variegation is due to the action of any of three recessive genes.

No evidence has been presented to show the relationship of the Corbett Refugee type of variegation to that reported, and attempts to make crosses of Corbett Refugee with the variegated types have failed in both the greenhouse and the field at Charleston, S. C. However, there is the probability that the variegation found in Corbett Refugee

and in U. S. No. 5 Refugee came originally from the same source, since over a period of years U. S. No. 5 Refugee had not previously yielded any variegation, and many lines of beans involving variegations derived from Corbett Refugee crosses were being carried at Greeley, Colo., at the time when the U. S. No. 5 Refugee, which later produced the variegation, was grown there. On many occasions it has been observed that crosses between Corbett Refugee and other varieties (notably Stringless Green Refugee and Brittle Wax) have given rise to progenies with very distinct variegation.

Variegations and chimeras have been found to behave variously in genetic studies, many of them being due more or less to plasmatic inheritance. These conditions have recently been reviewed by Sirks (*5*) and Jones (*2*).

Various seedsmen and breeders have reported that variegations in lines derived from Corbett Refugee behave peculiarly from a breeding standpoint and that from lines carrying chimeras they have been unable to isolate lines breeding true for the normal nonvariegated type. If the condition they reported is essentially the same as the one described in this paper and if they were selecting from an F_2 segregating population they could have expected only 1 plant out of 64 to be a pure normal; many of the variegations would have been obscured, since seedsmen normally make their selections 2 or 3 weeks after blooming time, when variegation is very difficult to detect.

It is suggested that seedsmen working with crosses involving such a variegation should make their observations several times during the growth of the plants so as to make sure that the plants selected do not show any variegation at any time. It is also suggested that many selections be made, probably a few hundred rather than a few dozen, in order to make sure that the desired normal type is selected.

CONCLUSIONS AND SUMMARY

The variegation treated in this paper is due to any one of three recessive factors, whereas the normal condition is due to the complementary action of three dominant genes giving in F_2 a ratio of 27 normal to 37 variegated for the cross of Black Valentine with the original variegated strain and the reciprocal cross. This was verified from F_1, F_2, and F_3 data as well as from some F_4 data for certain variable F_3 families. Intercrosses of the three types of variegated obtained from F_3 families segregating in 3:1 ratios gave nonvariegated F_1 plants and ratios of 9 normal to 7 variegated in F_2. These same three monofactorial variegated types crossed with the triple recessive gave all variegated in F_1 and F_2 generations.

The variegation is expressed over a wide range, from only a trace up to a very severe, semilethal condition. The range is approximately the same for variegated lines due to one, two, or three factors, but the average amount of variegation for three factors is more than for one or two, and the average for two more than for one. The relative order remained the same under favorable growing conditions in the field, under unfavorable conditions in the field, and under favorable growing conditions in the greenhouse. The best expression of variegation was obtained under greenhouse conditions, and the poorest under unfavorable growing conditions in the field.

Altogether 662 F_2 plants and 435 F_3 families were studied; the 180 F_3 segregating families consisted of 11,347 plants. In addition, some test crosses of various segregation types and some F_3 families were carried to F_4. The plants and families were about equally divided between the cross and its reciprocal. F_3 population chi squares indicated a significant deficiency of recessives in nearly all cases. This is to be expected, since in the very severe form the variegated condition does not have as great survival value as do the normal plants. Heterogeneity chi-square values indicated that in all cases homogeneous populations were combined for the F_3 chi-square tests.

LITERATURE CITED

(1) FISHER, R. A.
1936. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh and London.
(2) JONES, W. NEILSON.
1937. CHIMERAS: A SUMMARY AND SOME SPECIAL ASPECTS. Bot. Rev. 3: 545–562, illus.
(3) MATHER, K.
1938. THE MEASUREMENT OF LINKAGE IN HEREDITY. 132 pp., illus. London.
(4) REINKING, OTTO A., and WITHIAM, CHARLES G.
1940. DISEASE IN MOSAIC RESISTANT AND NON-RESISTANT GREEN REFUGEE BEANS. Canner 90 (9): 12–14, 28, illus.
(5) SIRKS, M. J.
1938. PLASMATIC INHERITANCE. Bot. Rev. 4: 113–131.
(6) WILSON, EDWIN B., and HILFERTY, MARGARET M.
1931. THE DISTRIBUTION OF CHI-SQUARE. Natl. Acad. Sci. Proc. 17: 684–688.
(7) ZAUMEYER, W. J.
1938. A HERITABLE ABNORMALITY OF BEANS RESEMBLING MOSAIC. Phytopathology 28: 520-522.

A MOSAIC DISEASE OF PRIMULA OBCONICA AND ITS CONTROL [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and JOHN T. MIDDLETON, *junior plant pathologist, California Agricultural Experiment Station* [2]

INTRODUCTION

A mosaic disease of *Primula obconica* Hance, grown extensively as a potted ornamental plant in commercial greenhouses in San Francisco, was first observed in August 1937. The incidence of the disease on very young to older seedling plants ranged from 5 to 25 percent and, because infected plants could not be marketed, serious financial losses were incurred.

The results of studies on transmission, experimental host range, and properties of the virus, as well as control of the disease, are presented in this paper.

REVIEW OF LITERATURE

That many cultivated species of the genus *Primula* are generally susceptible to virus infection is indicated in the literature. In addition to the mosaic diseases, the aster yellows, curly top, spotted wilt, and certain tobacco viruses may cause infection. Since this paper deals with a mosaic disease, only pertinent references are listed.

In Japan, Fukushi (*4*)[3] in 1932 and Hino (*6*) in 1933 recorded the occurrence of a mosaic disease on *Primula obconica*. The latter also observed a similar disease on *P. denticulata* Sm.

Smith (*13*) in England, in 1935, described a virus disease of the mosaic type on *Primula obconica* caused by cucumber virus 1. Later, Smith (*14*) stated that *Cucumis* virus 1 sometimes caused color breaking of the flowers and that other species of *Primula* were susceptible. *Cucumis* virus 1B, a strain, induced "a pronounced yellow and green mottling."

In Germany, Ludwigs (*7*) and Pape (*8*) described very briefly a virus disease of *Primula obconica* without apparently establishing the identity of the virus.

There are numerous brief references dealing with mosaic diseases of *Primula* spp. grown in greenhouses and out of doors, but the viruses were not identified. In 1926, Anderson et al. (*2*) mentioned the occurrence of a mosaic disease of *Primula* sp. in a Pennsylvania greenhouse, while Martin[4] recorded a similar disease affecting several hundred plants in a Michigan greenhouse which resulted in a total loss to the grower. Gram (*5*) and Yu (*16*) found mosaic-infected primroses grown out of doors. Flachs (*3*), White (*15*), and Pirone (*9*) also listed primroses as susceptible to mosaic-virus infection, but it is not clear whether they referred to species grown in greenhouses or in the field.

[1] Received for publication February 4, 1941.
[2] The assistance of nontechnical employees of the Federal Work Projects Administration Project No. 65-1-08-91 is acknowledged.
[3] Italic numbers in parentheses refer to Literature Cited, p. 679.
[4] MARTIN, G. HAMILTON. DISEASES OF FOREST AND SHADE TREES, ORNAMENTAL AND MISCELLANEOUS PLANTS IN THE UNITED STATES IN 1925. U. S. Bur. Plant Indus., [Mimeographed.] Plant Dis. Rptr. Sup. 50: 413-478, illus. 1926.

Under experimental conditions, Price (*10*) succeeded recently in transferring the alfalfa mosaic virus to *Primula obconica*. A description of the symptoms was not given.

SYMPTOMS OF THE DISEASE

Characteristic symptoms of the mosaic disease of *Primula obconica* plants are best expressed in greenhouses having a humid atmosphere

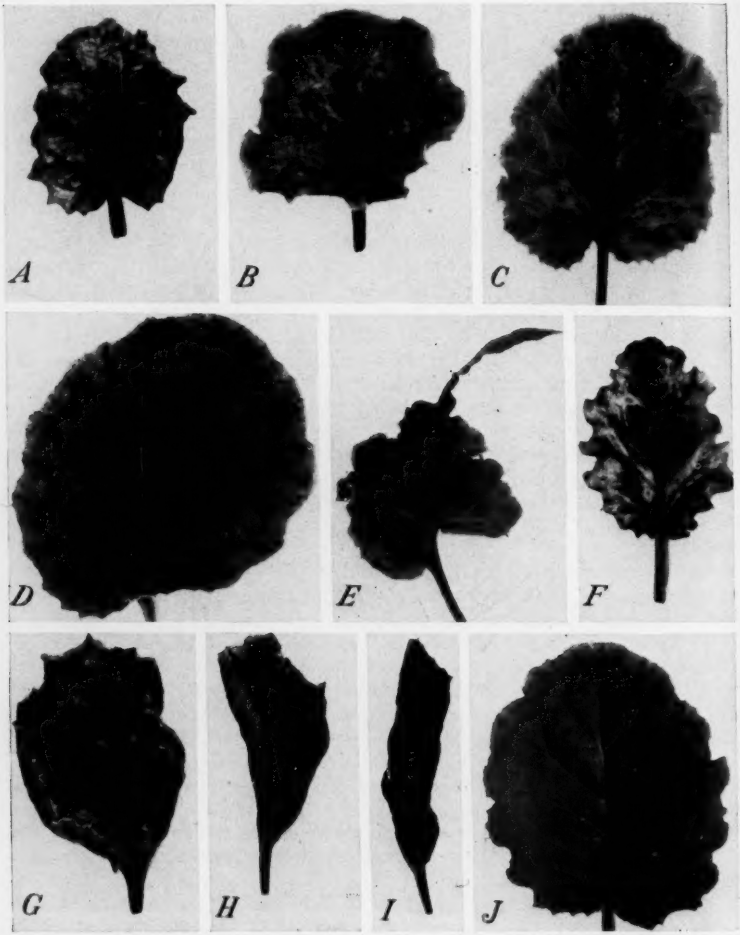

FIGURE 1.—Symptoms produced by a mosaic virus of *Primula obconica* on leaves of healthy *P. obconica* plants after mechanical inoculation in the greenhouse at 65° to 70° F.: *A*, *B*, Early symptoms, consisting of a systemic, yellow-green mottle; *C*, numerous, small, circular to irregular, raised, dark-green islands frequently occur between and adjacent to the veins on a mottled background; *D*, coarse, yellow-green mottle on leaf with flat, dark-green islands; *E*, a shoestring effect is sometimes produced, in addition to mottling and dark-green islands; late symptoms, consisting of *F*, rugosity of the leaves; *G*, *H*, *I*, cupping or upward rolling of the leaves; *J*, noninoculated control (healthy).

and air temperatures ranging from 65° to 70° F. Although the disease may affect young to middle-aged plants, it appears most commonly in young potted plants (three to five leaves). The first symptom consists of a systemic, coarse, yellow-green mottle of the leaves (fig. 1, *A*, *B*). With enlargement of the leaf area, the mottle becomes more conspicuous, consisting of numerous, dark-green, circular to irregular, raised islands (fig. 1, *C*), ranging in size from a mere fleck to 3 to 5 mm. in diameter, which are distributed between and adjacent to the veins and surrounded by nonraised or flat, light-green to yellow mottled areas (fig. 1, *A*, *B*). Infrequently, leaves of some infected plants show a coarse mottle with flat islands (fig. 1, *D*). Occasionally, also, the virus induces a shoestring effect at or near the tip of the leaf (fig. 1, *E*). These effects are in marked contrast to the appearance of a healthy leaf (fig. 1, *J*).

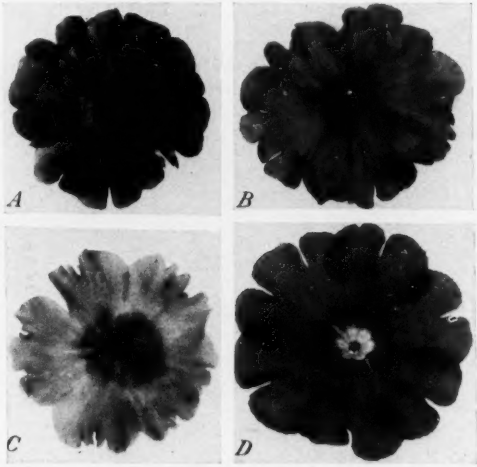

FIGURE 2.—Symptoms produced by a mosaic virus of *Primula obconica* on flowers of healthy *P. obconica* plants after mechanical inoculation in the greenhouse at 65° to 70° F.: *A*, *B*, *C*, Color breaking of flowers as indicated by irregular-shaped, white streaks interspersed among areas of normal pigmentation; *D*, noninoculated control (healthy).

With the onset of the disease, infected plants show a general chlorosis, marked stunting, and generally rugosity (fig. 1, *F*), with an upward curling or cupping of the leaves (fig. 1, *G*, *H*, *I*). Sometimes the cupping is so extreme that the edges of the leaf touch each other over the midrib. Occasionally the reverse condition holds, in that the center of the leaves becomes arched and the edges curl downward. The petioles and peduncles of infected plants are greatly shortened, while the flowers are usually much reduced in size. The petals show a conspicuous color breaking or variegation as indicated by irregular-shaped, white streaks interspersed among areas of normal pigmentation, which are more distinct near the base of the petals than at the edge (fig. 2, *A*, *B*, *C*), in contrast to the normal, healthy condition

(fig. 2, *D*). The most noticeable color breaking occurs on deep pink or lilac flowers, and frequently the pigments are almost totally destroyed. On lighter shades, it is difficult to detect any color change. Another symptom is a distinct, coarse mottle of the calyx. When infected plants reach maturity, they have usually only attained about one-fourth the normal size and cannot be marketed (fig. 3, *A*).

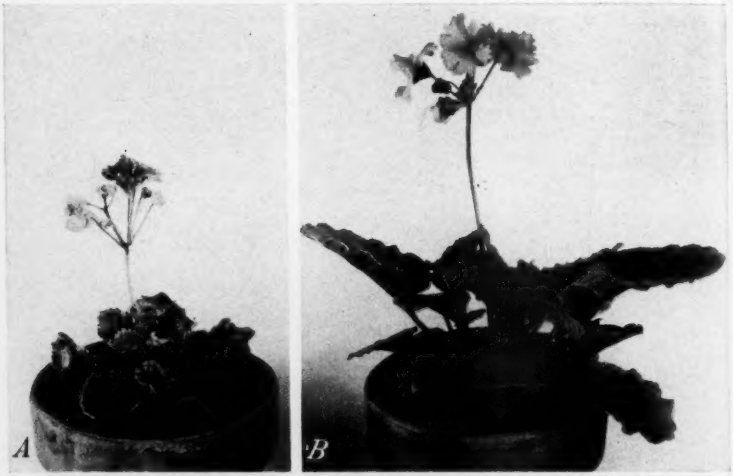

FIGURE 3.—Symptoms produced by a mosaic virus of *Primula obconica* on leaves of a healthy *P. obconica* plant after mechanical inoculation in the greenhouse at 65° to 70° F.: *A*, Diseased plant, showing dwarfing, mottling, and upward rolling of leaves and dwarfing of flowers; *B*, noninoculated control (healthy).

MATERIALS AND METHODS

Naturally infected *Primula obconica* seedlings were collected in a greenhouse in San Francisco, Calif., from which the mosaic virus used in these studies was obtained. The virus was transferred to healthy *P. obconica* plants, and transfers were made at monthly intervals. This plant species served also as the standard test plant for virus-recovery, virus-property, and insect-transmission studies.

All inoculations were made in a greenhouse where temperatures ranged from 65° to 70° F. Mechanical or juice inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*11*) and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

TRANSMISSION

The incubation period, as determined by numerous serial transfers of the virus by mechanical inoculation to healthy *Primula obconica* plants, ranged from 16 to 21 days.

Studies on insect transmission of this disease involved the testing of several species of aphids which are known to occur in local greenhouses, including the green peach aphid (*Myzus persicae* Sulzer) and the lily aphid (*Myzus circumflexus* Buckton). Noninfective

aphids were permitted to feed for 24 to 48 hours on infected plants and were then transferred to healthy plants. All attempts to transmit the virus by means of aphids were unsuccessful.

In excess of 20,000 *Primula obconica* seedlings (one to two leaves) in flats have been examined over a period of 3 years for evidence of virus infection, but with negative results. It must be assumed, therefore, that the virus is probably not seed-borne.

EXPERIMENTAL HOST RANGE

By means of mechanical inoculations, the *Primula obconica* mosaic virus was transmitted to two species within the same genus, namely, fairy primrose (*P. malacoides* Franch.) and Chinese primrose (*P. sinensis* Lindl.) and was later recovered from all infected plants. Infection was not obtained on any of the numerous species of plants belonging to different families as indicated by lack of visible symptoms on inoculated plants and by failure to recover the virus from them on healthy *P. obconica* plants. Numerous series of inoculations were made to Turkish tobacco (*Nicotiana tabacum* L.) and *N. glutinosa* L. with negative results.

This virus is apparently limited to the genus from which it was originally isolated. Somewhat analogous instances reported within recent years may be cited. According to Ainsworth (*1*), the green-mottle mosaic of cucumber (cucumber virus 3) and the yellow mosaic of cucumber (cucumber virus 4) occur in nature only on cucumber and are not transmissible to solanaceous plants. Severin and Freitag (*12*) succeeded in transferring the western celery mosaic virus only to species within the family Umbelliferae.

No infection was obtained by mechanical inoculation with the *Primula obconica* mosaic virus in 46 species of plants representing 42 genera in 23 families, as follows:

Begoniaceae:
- Fibrous-rooted begonia (*Begonia semperflorens* Link and Otto) var. Fire Sea

Boraginaceae:
- Forget-me-not (*Myosotis alpestris* Schmidt)

Campanulaceae:
- Canterbury bells (*Campanula medium* L.)

Caryophyllaceae:
- Sweet-william (*Dianthus barbatus* L.)

Chenopodiaceae:
- Spinach (*Spinacia oleracea* L.) var. Bloomsdale

Compositae:
- Romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.)
- English daisy (*Bellis perennis* L.)
- China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
- French marigold (*Tagetes patula* L.)
- Transvaal daisy (*Gerbera jamesonii* Hook.)
- Hybrid cineraria (*Senecio cruentus* DC.)

Cruciferae:
- Chinese cabbage (*Brassica pe-tsai* Bailey)
- Turnip (*B. rapa* L.) var. Purple Top White Globe
- Cabbage (*B. oleracea* L. var. *capitata* L.) var. Winter Colma
- Cauliflower (*B. oleracea* var. *botrytis* L.) var. February
- Radish (*Raphanus sativus* L.) var. White Icicle
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) var. Fiery Blood Red

Cucurbitaceae:
- Cucumber (*Cucumis sativus* L.) var. Arlington White Spine
- Pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) var. Zucchini

Euphorbiaceae:
- Castor-bean (*Ricinus communis* L.)

Gramineae:
- Corn (*Zea mays* L.) var. Golden Bantam

Leguminosae:
- Broadbean (*Vicia faba* L.)
- Cowpea (*Vigna sinensis* (Torner) Savi)
- Garden pea (*Pisum sativum* L.) var. Alderman

Lobeliaceae:
- Lobelia (*Lobelia hybrida* Hort.)

Papaveraceae:
- Oriental poppy (*Papave orientale* L.)

Primulaceae:
- *Primula auricula* L.
- Cowslip (*P. veris* L.)
- Florists' cyclamen (*Cyclamen indicum* L.)
- Poor-man's-weatherglass (*Anagallis arvensis* L.)

Ranunculaceae:
- Candle larkspur (*Delphinum cultorum* Voss)
- Persian buttercup (*Ranunculus asiaticus* L.)
- Poppy anemone (*Anemone coronaria* L.)

Resedaceae:
- Mignonette (*Reseda odorata* L.)

Rosaceae:
- *Geum chiloense* Balb.

Scrophulariaceae:
- Snapdragon (*Antirrhinum majus* L.)
- Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)

Solanaceae:
- Potato (*Solanum tuberosum* L.)
- Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
- Jimsonweed (*Datura stramonium* L.)
- Bell pepper (*Capsicum frutescens* L. var. *grossum* Bailey) var. California Wonder
- *Nicotiana glutinosa* L.
- Tobacco (*N. tobacum* L.) var. Turkish and White Burley

Tropaeolaceae:
- Garden nasturtium (*Tropaeolum majus* L.)

Umbelliferae:
- Celery (*Apium graveolens* L.) var. Golden Self Blanching

Verbenaceae:
- Garden verbena (*Verbena hybrida* Voss)

Violaceae:
- Pansy (*Viola tricolor* L.)

PROPERTIES OF THE VIRUS

The results of property studies of the *Primula obconica* mosaic virus are shown in table 1. Virus samples, consisting of 2 cc. of undiluted juice in small, thin-walled test tubes, were used in determining resistance to aging in vitro and inactivation temperature. In determining the tolerance to dilution, the virus was diluted with the required amount of distilled water. Young *P. obconica* plants were then inoculated mechanically with the different virus samples.

Samples of the *Primula obconica* mosaic virus were infectious after 24 hours aging in vitro at 22° C., but were inactivated after 48 hours. The virus produced infection after heating for 10 minutes at 48°, but was inactivated at 50°. A dilution tolerance of 1 to 10 was established. In each of the four trials, 16 noninoculated *P. obconica* plants served as controls and they remained healthy.

TABLE 1.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the Primula obconica mosaic virus*

[4 trials made with 16 plants each in all instances]

LONGEVITY IN VITRO, 22° C.

Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected
0	16	6	4	24	2	72	0
3	10	8	3	48	0	96	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected
40	15	48	14	55	0	65	0
45	12	50	0	60	0	(1)	16

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected
0	14	1:100	0	1:1,000	0	1:3,000	0
1:10	8	1:500	0	1:2,000	0	1:4,000	0

[1] Untreated.

DESCRIPTION OF THE PRIMULA OBCONICA MOSAIC VIRUS

Transmissible by mechanical inoculation with carborundum. Incubation period 16 to 21 days. Resistance to aging in vitro between 24 and 48 hours. Inactivation temperature between 48° and 50° C. for a 10-minute exposure. Tolerance to dilution approximately 1 to 10. Two additional species of *Primula* susceptible: *P. malacoides* and *P. sinensis*. Experimental host range apparently confined to this genus.

COMPARISON OF THE PRIMULA OBCONICA MOSAIC VIRUS WITH CERTAIN OTHER VIRUSES

As previously mentioned, Smith (*13*) has described a virus disease of *Primula obconica* in England caused by cucumber virus 1. During the course of the present studies, parallel series of inoculations were made simultaneously to healthy *P. obconica* plants with ordinary cucumber mosaic virus supplied by Dr. James Johnson of the Wisconsin Agricultural Experiment Station; an undescribed cucumber mosaic virus which occurs naturally in California; celery calico virus (*12*); and the mosaic virus of *P. obconica*[5] in order to determine whether the latter virus was similar to or identical with any of the aforementioned.

Ordinary cucumber mosaic virus and the undescribed California cucumber mosaic virus readily infected young *Primula obconica* plants, both inducing systemic infection. Symptoms caused by cucumber virus 1 consisted of a coarse mottle, with slight, upward cupping of the leaves, and a mild type of flower breaking. With the western cucumber mosaic virus, a coarse mottle, accompanied by

[5] The cucumber and celery viruses were kindly supplied by Drs. H. H. P. Severin and J. H. Freitag, Division of Entomology and Parasitology, University of California.

large, irregular-shaped, raised, dark-green islands, and severe flower breaking, occurred. In neither case were infected plants visibly retarded in growth. No infection resulted from inoculations with the celery calico virus. Considered as to symptoms, there can be little or no difficulty in differentiating between the mosaic virus of *P. obconica* and the other two viruses which infected this host.

Other plants which were tested with the four viruses include celery, cucumber, spinach, tomato, Turkish tobacco, and *Nicotiana glutinosa*. Again the *Primula obconica* mosaic virus failed to infect these plants, whereas infection was readily obtained with the other viruses.

As a result of these tests, it is concluded that the virus described in this paper is different from the three viruses with which it has been compared and the virus of *Primula obconica* described by Smith (*13*).

CONTROL OF THE DISEASE

Primula obconica plants showing mosaic infection were found on benches in different parts of the San Francisco greenhouses under observation, indicating a spread of the disease by some unknown insect vector. Attempts to locate other susceptible greenhouse plants which might serve as virus reservoirs and aphids which might conceivably act as vectors were unsuccessful.

In order to reduce, if not eliminate, further loss to growers as soon as possible, careful roguing of disease plants was practiced, and the greenhouses were fumigated weekly with nicotine dust. The incidence of the disease was quickly reduced, until at present it is believed that the disease has been entirely eradicated in these houses.

SUMMARY

A mosaic disease of *Primula obconica*, prevalent in greenhouses in San Francisco, is described.

The symptoms of the disease include a prominent leaf mottle, consisting of irregular-shaped, dark-green, raised or nonraised islands on a light-green to yellow background, with upward curling or cupping of the leaves. Occasionally a shoestring effect is induced at or near the tip of the leaf. The leaves, flowers, petioles, and peduncles are reduced in size. The petals of infected flowers show a conspicuous color breaking or variegation, while the calyx is mottled. Usually infected plants are severely stunted.

The *Primula obconica* mosaic virus is readily transmissible by juice inoculation, with carborundum. The incubation period ranges from 16 to 21 days. The green peach and lily aphids failed to transmit the virus under greenhouse conditions.

The virus retained its infectivity after aging for only 24 hours at 22° C. It is inactivated by heating for 10 minutes at 50° and has a dilution tolerance of 1 to 10.

The host range of the *Primula obconica* mosaic virus is limited to two additional species within the same genus, namely, *P. malacoides* and *P. sinensis*.

The disease has been eradicated by careful roguing of diseased plants and by frequent fumigation of the greenhouses.

LITERATURE CITED

(1) AINSWORTH, G. C.
1935. MOSAIC DISEASES OF THE CUCUMBER. Ann. Appl. Biol. 22: 55–67, illus.

(2) ANDERSON, PAUL J., HASKELL, ROYAL J., MUENSCHER, WALTER C., WELD, CLARA J., WOOD, JESSIE I., and MARTIN, G. HAMILTON.
1926. CHECK LIST OF DISEASES OF ECONOMIC PLANTS IN THE UNITED STATES. U. S. Dept. Agr. Dept. Bul. 1366, 112 pp.

(3) FLACHS, KARL.
1931. KRANKHEITEN UND PARASITEN DER ZIERPFLANZEN; EIN BESTIMMUNGS UND NACHSCHLAGEBUCH FÜR BIOLOGEN, PFLANZENÄRZTE, GÄRTNER UND GARTENFREUNDE. 558 pp., illus. Stuttgart.

(4) FUKUSHI, TEIKICHI.
1932. A CONTRIBUTION TO OUR KNOWLEDGE OF VIRUS DISEASES OF PLANTS IN JAPAN. Sapporo Nat. Hist. Soc. Trans. 12: [130]–141.

(5) GRAM, ERNST.
1933. PLANTESYGDOMME I DANMARK 1932. Tidsskr. for Planteavl 39: 453–506, illus. [In Danish. English summary, pp. 505-506.]

(6) HINO, IWAO.
1933. LIST OF PLANTS SUSCEPTIBLE TO MOSAIC AND MOSAIC-LIKE DISEASES. Miyazaki Col. Agr. and Forestry Bul. 5: [97]–111.

(7) LUDWIGS, [KARL].
1926. KRANKHEITEN AN ZIERPFLANZEN. Blumen- u. Pflanzenbau 41: 176–178, illus.

(8) PAPE, HEINRICH.
1932. DIE PRAXIS DER BEKÄMPFUNG VON KRANKHEITEN UND SCHÄDLINGEN DER ZIERPFLANZEN. 361 pp., illus. Berlin.

(9) PIRONE, P. P.
1939. DISEASES OF ORNAMENTAL PLANTS. N. J. Agr. Expt. Sta. Cir. 385, 80 pp., illus.

(10) PRICE, W. C.
1940. COMPARATIVE HOST RANGES OF SIX PLANT VIRUSES. Amer. Jour. Bot. 27: 530–541, illus.

(11) RAWLINS, T. E., and TOMPKINS, C. M.
1936. STUDIES ON THE EFFECT OF CARBORUNDUM AS AN ABRASIVE IN PLANT VIRUS INOCULATIONS. Phytopathology 26: 578–587, illus.

(12) SEVERIN, HENRY H. P., and FREITAG, JULIUS H.
1938. WESTERN CELERY MOSAIC. Hilgardia 11: [493]–558, illus.

(13) SMITH, KENNETH M.
1935. A VIRUS DISEASE OF PRIMULA OBCONICA AND RELATED PLANTS. Ann. Appl. Biol. 22: 236–238, illus.

(14) ———
1937. A TEXTBOOK OF PLANT VIRUS DISEASES. 615 pp., illus. Philadelphia.

(15) WHITE, RICHARD P.
1931. DISEASES OF ORNAMENTAL PLANTS. N. J. Agr. Expt. Sta. Cir. 226, 98 pp., illus.

(16) YU, T. F.
1939. A LIST OF PLANT VIROSES OBSERVED IN CHINA. (Phytopath. Note) Phytopathology 29: 459–461.

RELATION BETWEEN WETTING POWER OF A SPRAY AND ITS INITIAL RETENTION BY A FRUIT SURFACE[1]

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INTRODUCTION

The wetting power of a spray solution is a very important factor in its efficiency as a contact poison or as a stomach poison. In the field of contact sprays this has been clearly recognized by a number of workers (*1*, *8*, *9*, *11*)[2], who have presented logical explanations of their results and of the physicochemical phenomena involved. In earlier papers (*2*, *3*, *4*, *5*) the author has summarized some of this work and has developed the use of the spreading coefficient on mineral oil as a convenient quantitative measure of wetting power. The spreading coefficient, S, of an aqueous solution with respect to a mineral oil is defined by the equation $S = T_B - T_A - T_{AB}$, where T_B is the surface tension of the oil, T_A is the surface tension of the aqueous solution, and T_{AB} is the interfacial tension.

In the field of stomach poisons and protective fungicides, which function by means of the deposit that remains after evaporation of the liquid medium, the factors involved have been less clearly recognized. Working with oil-emulsion sprays, Smith (*10*) has found that increased amounts of emulsifier produce sprays which wet the surfaces better but which deposit less oil, and he has advanced a working hypothesis to explain this phenomenon. However, his explanation does not clearly point out, or take into account, the fact that when the spray mixture wets the surface poorly a much greater volume may be retained on the sprayed surface. This factor alone may account for the observed increase in the deposition of insecticide when little or no emulsifier is used. The author (*5*) has briefly commented on the factors involved in the application of spray mixtures of this type and has pointed out that maximum efficiency may be associated with relatively high surface and interfacial tensions, i. e., with a low spreading coefficient. Fajans and Martin (*6*, *7*) have recently reported work in this field, in which they have determined solid-liquid contact angles as a measure of wetting power.

Statements are occasionally made that an excess of wetter causes excessive run-off and a reduced deposit of insecticidal material on the fruit and foliage, or, conversely, that the addition of a wetter produces better wetting and adherence and an increased deposit of insecticidal material. Obviously, in any given case many factors are involved besides the specific nature of the surface to which the spray is being applied. Such factors include the wetting agent, the influence of other constituents of the spray mixture, and the water supply. The object of the present work has been to determine whether there is a definite relation between the wetting power of

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[2] Italic numbers in parentheses refer to Literature Cited, p. 685.

the spray mixture, as measured by its spreading coefficient on mineral oil, and the volume of spray mixture retained by fruit and foliage. If so, the problem can possibly be simplified to the consideration of but two factors, (1) the surface being sprayed and (2) the spreading coefficient of the spray mixture.

EXPERIMENTAL METHOD

The general plan of the experiments was to rotate an apple, while spraying it with a spray solution of known spreading coefficient, until run-off commenced, and then to determine the volume of the spray solution retained on the apple. Variation in wetting power was produced by the addition of different amounts of a wetting agent, and the spreading coefficients were determined as previously described (*5*). Two series of tests were made in which two distinct methods for determining the volume of retained spray solution were used. An interval of several months separated the two series. As the spreading coefficient associated with a definite retention would be expected to vary with the type of fruit, its maturity, and possibly other factors, these experiments are regarded as illustrative only.

For rotating the apple during the spraying the stem was tied to a string dropped from the vertical shaft of a variable-speed laboratory stirrer, which was operated at about 30 revolutions per minute. The spray solution was applied with a small lacquer-spraying nozzle attached to the laboratory air supply, which was kept at a constant distance from the apple and was manipulated in as nearly uniform a manner as possible. The spray thus produced was a rather fine mist, and it is possible that a coarse, driving spray would give somewhat different results.

TESTS WITH SPRAYS CONTAINING SODIUM BICARBONATE AND WETTING AGENT

In the first series of tests the spray liquid was a solution of sodium bicarbonate, approximately N/2, containing varying amounts of a wetting agent (No. 1). Measurements were made of the surface tension and the spreading coefficient on mineral oil of most of the spray solutions. With the apple rotating, spraying was begun and continued until a distinct run-off occurred. Spraying was then discontinued, rotation of the apple stopped, and the spray solution retained by the apple was quickly washed into a beaker with distilled water. The sodium bicarbonate thus recovered from the apple was titrated with standard hydrochloric acid, methyl orange being used as indicator.

The titer of the spray mixture being known, a simple calculation gave the volume of spray solution retained by the apple, upon the assumption that there was no concentration of the solution by evaporation. Each of the uncorrected values thus obtained was multiplied by the constant factor 0.55, to make an approximate correction for the increase in concentration of the solution due to evaporation during the spraying process. This factor was determined by weighing the apple before and after spraying with a mixture containing no wetting agent and comparing the volume of retained spray thus found with the volume found by titration.

Table 1 and figure 1 present the results of these experiments, together with the calculated values of the relative retention, which is here defined as the ratio of the volume of spray retained to the volume retained when the spray liquid is distilled water.

TABLE 1.—*Variation of wetting properties and initial retention with the concentration of wetter No. 1 in an approximately N/2 solution of sodium bicarbonate*

Concentration of wetter		Spray retained		Relative retention	Surface tension	Interfacial tension	Spreading coefficient
		Titration	Corrected				
Percent by weight	*Log of grams per cubic meter*	*Cubic centimeters*	*Cubic centimeters*		*Dynes per centimeter*	*Dynes per centimeter*	*Dynes per centimeter*
1.000	4.000	0.51	0.28	0.14	29.2	1.1	+0.2
.500	3.699	.57	.31	.16			
.250	3.398	.50	.28	.14			
.125	3.097	.58	.32	.16	29.2	.7	+.6
.0625	2.796	.58	.32	.16	28.8	.8	+.9
.0313	2.496	.64	.35	.17			
.0156	2.193	.83	.46	.23	29.2	1.7	−.4
.00781	1.893	1.02	.56	.28	31.3	3.1	−3.9
.00391	1.592	1.58	.87	.44	32.4	4.7	−6.6
.00195	1.290	2.24	1.23	.62	34.8	7.3	−11.6
.000977	.990	2.93	1.61	.81	37.4	13.3	−20.2
.000493	.693	3.54	1.95	.98	38.6	14.3	−22.4
.000247	.393	3.36	1.85	.93			

TESTS WITH SPRAYS CONTAINING DISTILLED WATER AND WETTING AGENT

In the second series of tests the spray mixture contained no sodium bicarbonate, and was simply an aqueous solution of a wetting agent (No. 2). These experiments were similar to those of the first series except that a different method was used for determining the volume of spray solution retained by the apple. The method used here was to remove mechanically and collect the retained liquid by quickly absorbing it into a tuft of absorbent cotton, the cotton being weighed in a glass-stoppered container before and after adsorption of the liquid.

These results are presented in table 2 and figure 1, together with values of the relative retention, as explained above.

TABLE 2.—*Variation of wetting properties and initial retention with the concentration of wetter No. 2 in distilled water*

Concentration of wetter		Spray retained	Relative retention	Surface tension	Interfacial tension	Spreading coefficient
Percent by weight	*Log of grams per cubic meter*	*Cubic centimeters*		*Dynes per centimeter*	*Dynes per centimeter*	*Dynes per centimeter*
1.000	4.000	0.62	0.24	33.8	1.8	−5.1
.500	3.699	.53	.21	32.9	1.3	−3.7
.200	3.301	.80	.31	33.6	1.8	−4.9
.100	3.000	1.28	.50	35.7	4.9	−10.1
.0500	2.699	1.74	.68	39.7	8.1	−17.3
.0200	2.301	1.99	.78	39.9	12.6	−22.0
.0100	2.000	2.61	1.02	42.3	18.6	−30.4
.0000	--------	2.55	1.00	72.0	42.6	−84.1

DISCUSSION

The curves presented in figure 1 indicate that there is a close relationship between the spreading coefficient and the relative retention. The curve for series 1 shows that a relative retention of 0.50 is obtained when the concentration of wetter is 35 gm. (antilog of 1.54) per cubic meter. The curve for series 2 shows the same relative retention at a concentration of 1,050 gm. (antilog of 3.02) per cubic meter. Thus, for the same relative retention the concentration of wetting agent is 30 times as great in series 2 as in series 1. At the same concentrations the corresponding spreading coefficients are −8 and −9. These values agree well, considering the nature of the experiments and the time interval separating the two series.

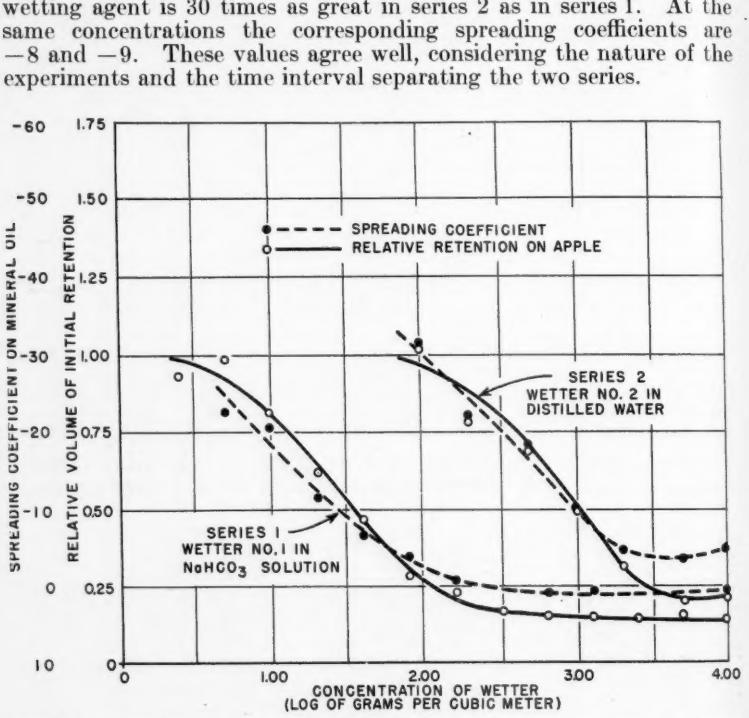

FIGURE 1.—Wetting properties as related to the concentration of wetting agents in spray solutions.

Iu figures 2 the values of relative retention are plotted against the values of spreading coefficient, and a line has been drawn through the points by inspection. The points representing series 1 lie rather consistently higher than the points representing series 2, and this deviation may possibly be significant. The deviation may be due to a difference in the character of the fruit surfaces used in the two series, or it may be simply a manifestation of the fact that the spreading coefficient is not a perfect measure of wetting power. However, all the points considered together show a good correlation between relative retention and spreading coefficient; i. e., the lower the spreading

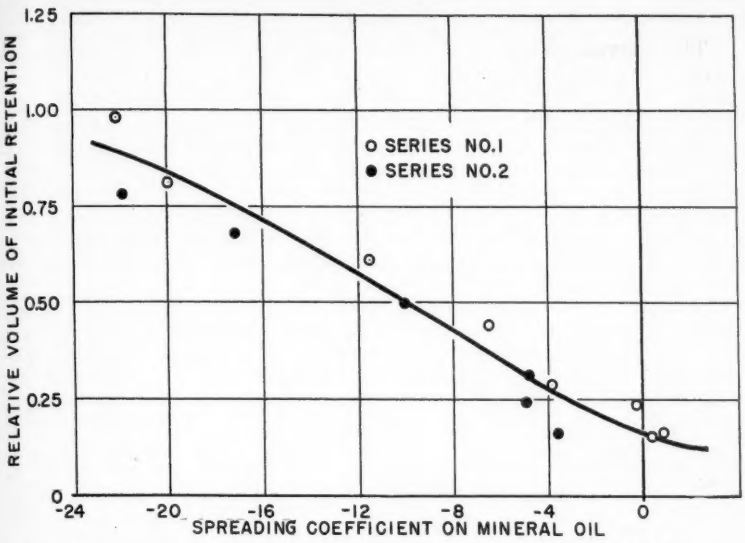

FIGURE 2.—Spreading coefficient as related to the initial retention of spray solutions.

coefficient the greater the volume of spray retained. A similar correlation may be expected to occur in the case of other fruit and foliage surfaces. It is suggested that the spreading coefficient on a reference mineral oil may be used as a practical measure of the wetting properties of aqueous spray solutions.

SUMMARY

The relation between the wetting power of a spray mixture and its retention on the sprayed surface has been investigated by spraying a rotating apple with spray solutions of varying wetting power, as measured by their spreading coefficients on mineral oil, and determining the retention of spray mixture at the point of run-off. Plots of the spreading coefficients against the corresponding retentions of spray mixture show that there is a close relationship between these values.

LITERATURE CITED

(1) COOPER, W. F., and NUTTALL, W. H.
1915. THE THEORY OF WETTING, AND THE DETERMINATION OF THE WETTING POWER OF DIPPING AND SPRAYING FLUIDS CONTAINING A SOAP BASIS. Jour. Agr. Sci. [England] 7: [219]–239, illus.

(2) CUPPLES, H. L.
1935. WETTING AND SPREADING PROPERTIES OF AQUEOUS SOLUTIONS. OLEIC ACID-SODIUM HYDROXIDE MIXTURES. Indus. and Engin. Chem., Indus. Ed. 27: 1219–1222, illus.

(3) ———
1936. WETTING AND SPREADING PROPERTIES OF AQUEOUS SOLUTIONS. OLEIC ACID-SODIUM CARBONATE MIXTURES. Indus. and Engin. Chem., Indus. Ed. 28: [60]–62, illus.

(4) CUPPLES, H. L.
1936. WETTING AND SPREADING PROPERTIES OF AQUEOUS SOLUTIONS. MIXTURES OF OLEIC ACID WITH POTASSIUM HYDROXIDE, POTASSIUM CARBONATE, AND AMMONIA. Indus. and Engin. Chem., Indus. Ed. 28: 434–436, illus.

(5) ———
1937. WETTING AND SPREADING PROPERTIES OF AQUEOUS SOLUTIONS. MIXTURES OF SODIUM HYDROXIDE WITH N-CAPROIC, N-CAPRYLIC, N-CAPRIC, LAURIC, MYRISTIC, AND PALMITIC ACIDS. Indus. and Engin. Chem., Indus. Ed. 29: 924–926, illus.

(6) FAJANS, E., and MARTIN, H.
1937. THE INCORPORATION OF DIRECT WITH PROTECTIVE INSECTICIDES AND FUNGICIDES. II. THE EFFECTS OF SPRAY SUPPLEMENTS ON THE RETENTION AND TENACITY OF PROTECTIVE DEPOSITS. Jour. Pomol. and Hort. Sci. 15: 1–24.

(7) ——— and MARTIN, H.
1938. THE INCORPORATION OF DIRECT WITH PROTECTIVE INSECTICIDES AND FUNGICIDES. III. FACTORS AFFECTING THE RETENTION AND SPRAY RESIDUE OF EMULSIONS AND COMBINED EMULSION–SUSPENSIONS. Jour. Pomol. and Hort. Sci. 16: 14–38 illus.

(8) MOORE, WILLIAM.
1918. OBSERVATIONS ON THE MODE OF ACTION OF CONTACT INSECTICIDES. Jour. Econ. Ent. 11: 443–446.

(9) O'KANE, W. C., WESTGATE, W. A., GLOVER, L. C., and LOWRY, P. R.
1930. SURFACE TENSION, SURFACE ACTIVITY, AND WETTING ABILITY AS FACTORS IN THE PERFORMANCE OF CONTACT INSECTICIDES. STUDIES OF CONTACT INSECTICIDES I. N. H. Agr. Expt. Sta. Tech. Bul. 39, 42 pp., illus.

(10) SMITH, RALPH H.
1932. THE TANK-MIXTURE METHOD OF USING OIL SPRAY. Calif. Agr. Expt. Sta. Bul. 527, 86 pp., illus. [Revised 1933.]

(11) WILCOXON, FRANK, and HARTZELL, ALBERT.
1931. SOME FACTORS AFFECTING THE EFFICIENCY OF CONTACT INSECTICIDES. I. SURFACE FORCES AS RELATED TO WETTING AND TRACHEAL PENETRATION. Boyce Thompson Inst. Contrib. 3: 1–12, illus.

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